

NEW CONCEPTS IN THE PATHOPHYSIOLOGY OF MACULAR DISEASE:
MORPHOLOGICAL RESPONSES IN THE CHORIOCAPILLARIS AND
BRUCH'S MEMBRANE.

Volume I

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Abstract

Macular disease is the commonest cause of untreatable visual handicap in the Western world. Considerable research has been done on the epidemiology and natural history of the disease, and on the anatomy, physiology and pathology of the structures involved. The literature on the subject is reviewed in this thesis.

The study is divided into three parts. In the first clinico-pathological correlation of three different types of chorioretinal disease, namely age related macular degeneration, retinitis pigmentosa and a form of glomerulonephritis associated with a choroidopathy are presented.

In the second part, experimental studies aimed to investigate the cellular pathological responses in Bruch's membrane, with emphasis on the role of the pericytes of the choriocapillaris, are described.

In the third part two clinical studies are discussed. Firstly, a group of patients with age related macular degeneration are reviewed in the light of concepts developed in the preceding two sections. In the second, a group of patients with a type of glomerulonephritis and a previously undescribed choroidopathy was studied, substantiating a hypothesis developed earlier in the thesis that the choriocapillaris - Bruch's membrane - retinal pigment epithelium (RPE) complex has features in common with the renal glomerulus both anatomically and in pathophysiological responses.

It is concluded that there is a cellular response to lesions of Bruch's membrane and the RPE, in which pericytes, circulating monocytes, choriocapillary endothelial cells and RPE cells participate. The parallel drawn between the eye and the glomerulus offers the opportunity of regarding both sites in a new light offered by the other discipline and suggesting avenues for further collaborative research.

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I collaborated with other workers on parts of this thesis, and they are acknowledged appropriately.

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My husband, James, helped me to prepare reference lists, and goaded me into completing this project.

Declaration

I, Josephine Duvall-Young declare that I prepared this thesis, and that all the work is my own, with the exception of Chapter II part 2, which has been published as a paper in collaboration with Dr. N.M. McKechnie, Professor W.R. Lee, Dr. S. Rothery and Professor J. Marshall and Chapter II part 3 in which I collaborated with N.M. McKechnie and Dr. M.K. McDonald. I was the principal investigator for both studies.

The experimental work was done in the laboratories of Dr. M.O.M. Tso, University of Illinois, Chicago, under his supervision (Chapter III).

I have collaborated with Dr. C. Short and Dr. M.F. Raines in studying a group of patients with glomerulonephritis (Chapter IV part 2) but I was principal investigator.

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Introduction

The chorioretinal junction structures, namely, the choriocapillaris, Bruch's membrane, and retinal pigment epithelium (RPE), are the site of pathological responses to injury and disease, both acquired and congenital, particularly at the macula. The nature and aetiology of these responses are ill understood, but the end results of them are manifest as the major causes of blindness in the developed world. In the first chapter of this thesis, the existing literature covering the structure, function and pathological responses will be reviewed and related to current theories of the pathophysiology of disease processes. In the second, third and fourth chapters, original work directed at advancing our understanding of disease will be presented, with specific attention to the cellular responses in Bruch's membrane. The results of these studies indicate the direction of further research, with possible therapeutic implications.

Chapter I

Literature Review

1. Anatomy

The layers to be considered are the choriocapillaris, Bruch's membrane and the retinal pigment epithelium (Fig. I 1).

Choriocapillaris

The choriocapillaris is the capillary bed of the choroid, the vascular coat of the eye. It was first described by Hovius (532) and later named by Eschricht (278). It forms the innermost of the four layers of the choroid, the outermost being the lamina suprachoroidea, the next the thick layer of the large vessels, then a layer of smaller vessels. Early this century, detailed descriptions of the anatomy were already published (686,989) and the choriocapillaris was recognized as a continuous network of capillaries lining the inner aspect of the choroid (1161). Wudka and Leopold have made an extensive historical literature review (1210). More recently, studies of the anatomy based on injected specimens, flat preparations, histochemistry, and electron microscopy have been published. The technique of neoprene cast preparation was described by Ashton (38) and further studied by Wybar (1214,1215), demonstrating a network of arterioles and venules (38) with a segmental pattern of supply to the choriocapillaris (1215). Friedman and

Figure I₁

Light micrograph showing normal anatomy of chorioretinal juncture, p photoreceptor outer segments. rpe - retinal pigment epithelium, bm Bruch's membrane, cc Choriocapillaris. Endothelium cell, large arrowhead. Pericyte small arrowhead (Stain Mallorrey's Blue x 1000).

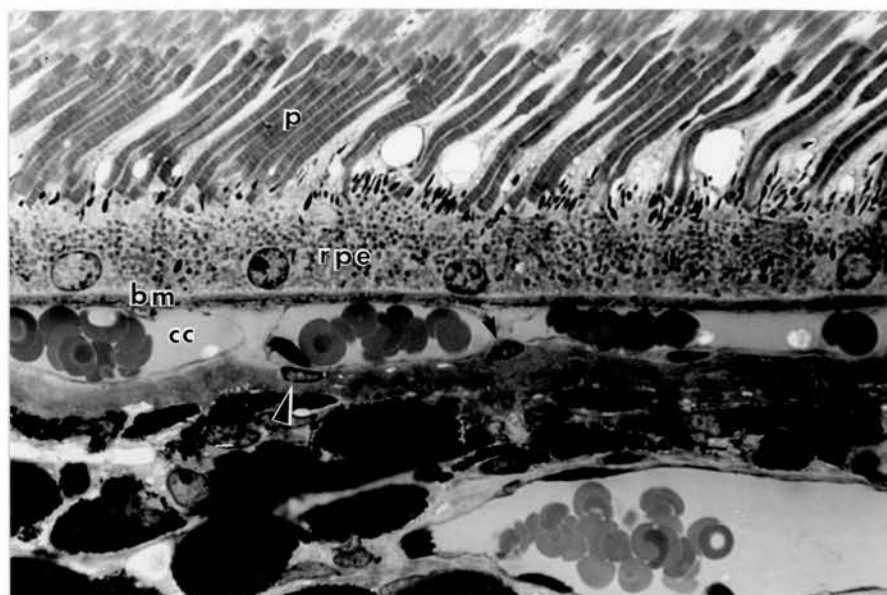


Figure 1 1

colleagues (371) described the technique for flat preparations of the choroid and characterised the choriocapillaris as a network of capillaries in a single plane. Although Ring and Fujino (956) believed the choriocapillaris to be a freely anastomosing vascular bed, other investigators now support the segmental pattern proposed by Ashton (38) and Wybar (1215) and also show that the capillaries are distributed in lobules of the order of 600 μ in diameter(46,247,480,959,1112,1184A,1233). It appears that the capillary bed is different in the macular area, where the supply of blood to the lobules is not through end arteries (493,1150) allowing a degree of anastomosis to occur, while more peripherally, the lobular pattern is more clearly defined (1148).

The structure of the lobules is disputed. Hayreh (480) and others (30,1233) demonstrated lobules with a central arteriole and peripheral venules. Hayreh (480) derived his concept of the choriocapillary lobule from fluorescein angiographic studies but he may have misinterpreted vascular leakage as vascular filling (979). In the studies of Yoneya and Tso (1232,1233) the resolution in the casting technique was insufficient to differentiate arterioles from venules. Although the question is not completely resolved (483), the body of evidence suggests that the lobule is centred on a venule with peripheral arterioles. Krey (646) using alkaline phosphatase staining on flat preparations, and Woodlief and Eifrig (1206) in cast preparations showed lobules with a central venule and peripheral arterioles. This

structure fits with the number of arterioles relative to venules found in sections, and also with physiological measurements of blood flow.

Attempts have been made to explain the pattern of distribution of choroidal and retinal disease on morphological variations in the choriocapillaris (371,623,1215) the majority of diseases having a predilection for the posterior pole. However, the structure of the choroid is very similar in different parts of the eye. At the posterior pole, the capillaries are of smaller diameter and are more closely packed than anteriorly (623,956,1031,1112,1215) but there is no difference between the nasal and temporal side. However, a number of chorioretinal diseases are seen almost exclusively on the temporal side of the posterior pole, that is, in the macular area.

The capillaries of the choroid have a larger diameter than elsewhere in the body, being approximately 20μ in diameter in the macular region and up to 50μ more anteriorly (387,623,956,1030,112,1215). They are separated from each other by connective tissue containing elastic and collagen fibers. In the connective tissue of the choroid are found mast cells, plasma cells, and macrophages, as well as fibroblasts, melanocytes (302,517,1197), nerves (1203) and ganglion cells (659), but these are not present in the stroma of the choriocapillaris itself (1091).

The capillary wall is made up of fenestrated endothelial cells, the cell body of which is almost always on the scleral aspect of the capillary (38,69,189,296,473,692,812,942,1091,1100) (Fig. I 2). On the inner aspect, the endothelial cells are thin with diaphragmed fenestrae (387,556,762,786,812,875,1062,1066,1089,1101). The majority of the fenestrae are on the retinal side of the capillaries (69) and are more frequent at the posterior pole (296,1089). They are particularly well demonstrated using the freeze etch technique for preparation for electron microscopy (690). There is a central thickening in the diaphragm (387,812,1062) as is described in capillaries of the renal glomerulus (953). The endothelial cytoplasm contains numerous pinocytotic vesicles and coated vesicles, (837) but few other organelles, those being confined to the cell body (296,387) (Fig I 3,4). The endothelial cells are joined by zonulae occludentes (762,1062,1066), and the whole capillary is surrounded by basement membrane which also envelops the pericytes (387,1091).

Pericytes were considered not to exist in the choriocapillaris (692,956). Ashton, in a detailed description of the anatomy of the choriocapillaris, made no mention of these cells (38). They were not recognized because in normal tissue, they are usually only represented in sections by small cytoplasmic processes with few organelles other than pinocytotic vesicles and indeterminant granules, seen more frequently on the scleral than on the retinal aspect of the capillary .

Figure I₂

A. Electron micrograph from a 79 year old human Bruch's membrane (BM) contains heterogeneous collagenous tissue, including banded basement membrane (b) in the inner collagenous zone. The choriocapillaris (CC) is enveloped in basement membrane (large arrowheads). The endothelium on the inner aspect is fenestrated (small arrowheads). Pericyte cell processes (P) are present on the outer aspect of the choriocapillary endothelium (E). Note a few curvilinear membrane profiles in the extracellular material on the scleral aspect of the choriocapillaris (small arrow) (x 16,000).

B. Electron micrograph of choriocapillaris and Bruch's membrane (BM) shows aging changes with accumulation of vesicular structures. Fenestration of the endothelium of the choriocapillaris (CC) are seen on the retinal, and to a lesser extent, the scleral aspect of the capillary (arrowheads). Pericyte processes (P) enveloped in basement membrane are seen on the scleral aspect of the capillary. Melanocytes (M) and other unidentified cells (S) are seen in the stroma of the choroid. A monocyte with its typical indented nucleus ^{and numerous cytoplasmic organelles} includ-

ing *rough* endoplasmic reticulin, mitochondria and lysosomes is present in the choriocapillary lumen. (x 12,000).

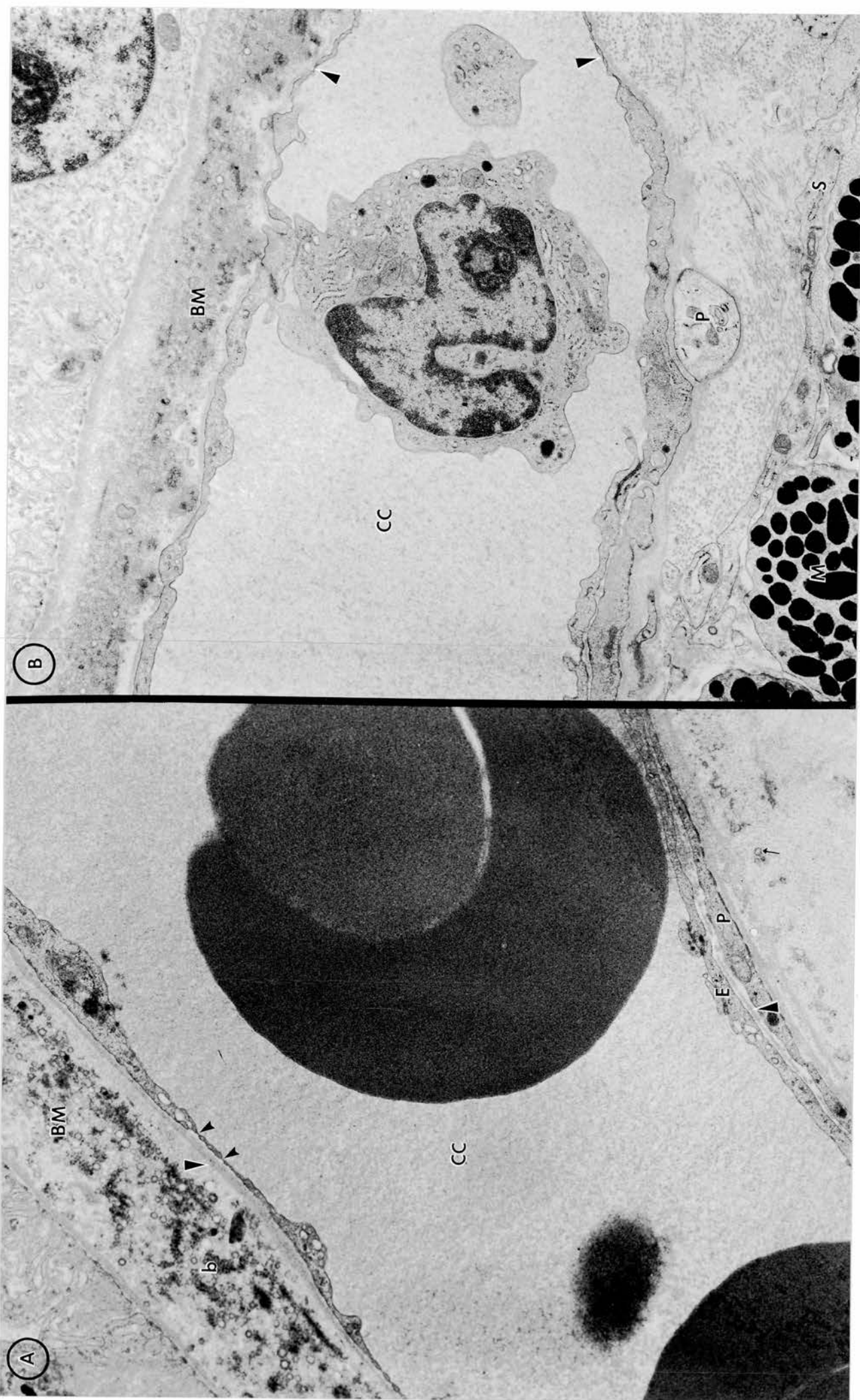


Figure 1 2

Figure 13

Electron micrograph from a 79 year old human. Note the basal infoldings (large arrows) of the RPE (RPE). The elastic layer (e) of Bruch's membrane is prominent. The fenestrations of the choriocapillaris (large arrowheads) are seen on the inner aspect of the choriocapillaris (CC). The outer wall is made up of the cell bodies of an endothelial cell (E) and a pericyte (P). Both cells are enveloped in basement membrane (small arrowheads) focal densities are a prominent feature of the pericyte (small arrows) ($\times 23,000$).



Figure 1 3

Figure 14

Electron micrograph showing the same cells as in Figure 13 at higher power. Pinocytotic vesicles (small arrowheads) and basement membrane (large arrowheads) are seen in relation to both the endothelial cell (E) and the pericyte (P). Cytoplasmic membrane focal densities (arrows) are a prominent feature of the pericytes ($\times 23,000$).

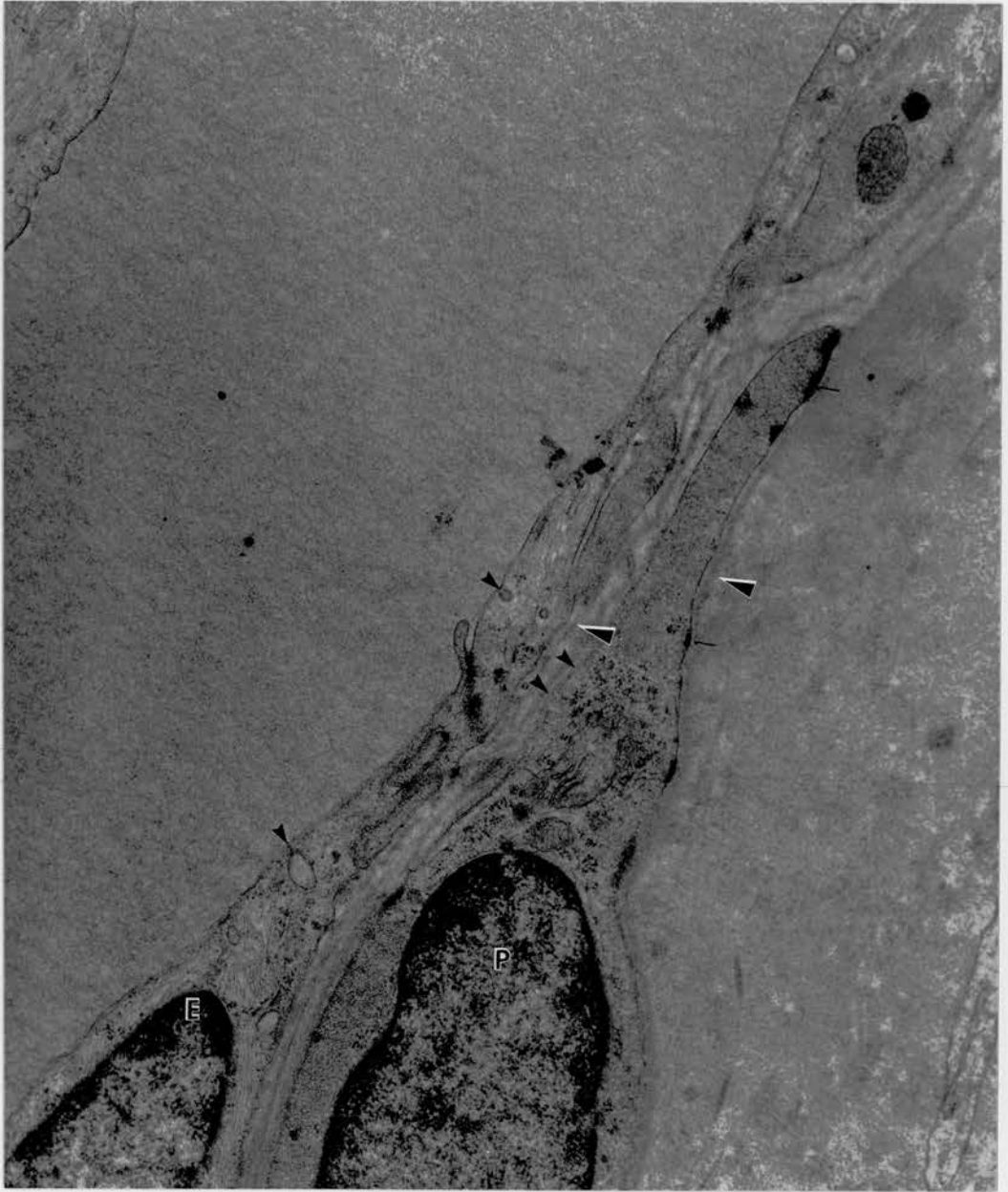


Figure 1 4

The cell body is invariably on the scleral aspect (20b). By light microscopy, the cells are insignificant and can only clearly be recognized by electron microscopy. Cells which are probably pericytes were illustrated by Friedman and coworkers, in flat preparations, but were not recognized by the authors (371). Wolter(1199), using a silver staining technique, made the first detailed description of pericytes in the choriocapillaris, demonstrating the fine branching structure of the cytoplasmic processes. Schaly (1000) had earlier described the pericytes of the choriocapillaris of the eye, but his descriptions are vague and illustrations poor.

Morphometric analysis of the relationship of pericytes to endothelial cells in the vascular beds of the uvea and of the retina of rats showed that pericytes are less frequently present in sections of the choriocapillaris than in the retinal and anterior uveal capillaries (763,1111). They are intimately related to the endothelial cells, forming part of the luminal surface of the capillary more often in the choriocapillaris than in other circulations. They form gap junctions with endothelial cells on the scleral side of the capillaries (762,1066). These may be representative of metabolic coupling of neighboring cells (419), but the significance of the relationship of the cells to each other is unknown.

Bruch's membrane

In 1844, Bruch first described a "structureless membrane" between the retina and choroid (121). By light microscopy, it was described as a two layered structure with a cuticular layer associated with the retinal pigment epithelium and an elastic layer. Wolfrum (1196) described three layers, the third being a collagenous zone between the other two. Detailed ultrastructural studies reveal five layers; the basement membrane of the retinal pigment epithelium, the inner collagenous zone, the elastic lamina, the outer collagenous zone and the basement membrane of the choriocapillaris (387,518,834,835,878,1159). The collagenous zones are made up of randomly arranged fibrils divided by clumps of elastin of the elastic layer which is fenestrated (310,698,834). The inner collagenous zone is of the order of 1.5μ in thickness, and the outer 0.7μ . The elastic layer is about 0.8μ thick, composed of elastic fibrils of varying size forming a meshwork (143) with collagen fibrils from the two collagenous zones passing through the interstices (1255) (Fig I 5). Oblique sectioning shows the membrane have a sieve-like appearance (518). Posteriorly, the collagenous and elastic layers are more dense than anteriorly (387) and Bruch's membrane is thicker. The retinal pigment epithelium is attached to its basal lamina by hemidesmosomes (800) but there is no fine attachment between the basal lamina of the retinal pigment epithelium and the inner collagenous zone (431,834). On the outer aspect, Bruch's membrane is inseparably

Figure 1s

Diagram of Bruch's membrane showing the 5 layers
recognised ultrastructurally.

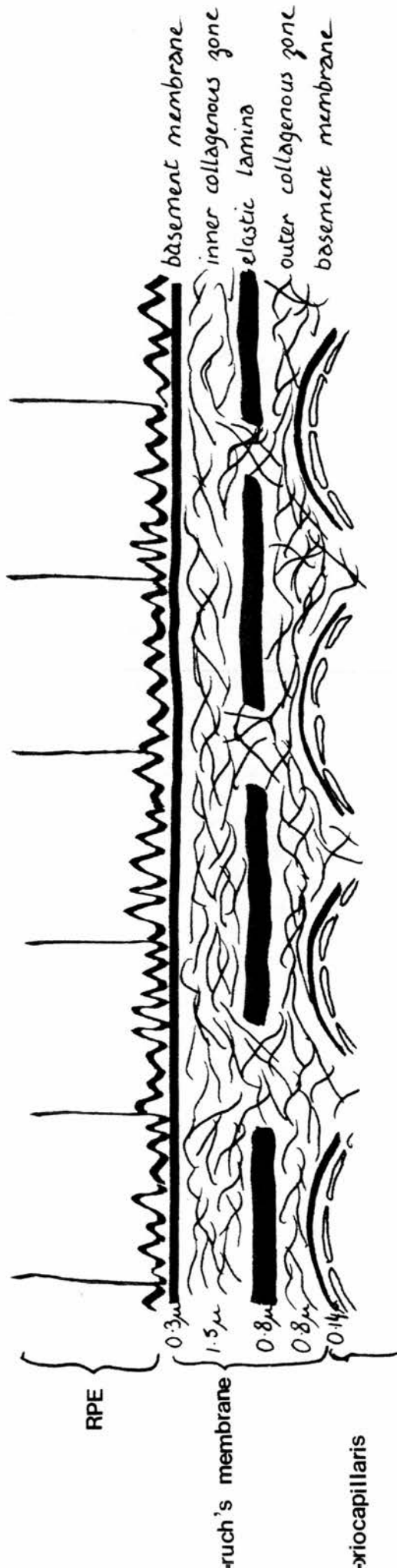


Figure 1 s

attached to the collagenous meshwork of the choriocapillary stroma (1198). The basement membrane of the retinal pigment epithelium is of the order of 0.3μ thick, and of the choriocapillaris 0.14μ . The former is continuous while the latter is discontinuous at the intercapillary zones (1073,1255).

Retinal pigment epithelium

Jones described the anatomy of the pigment epithelium in 1833 (577) reviewing earlier studies dating back to Carlo Mondini of Bologna in 1790. The first comprehensive description of the retinal pigment epithelium was by Bruch (121).

The retinal pigment epithelium has been studied by conventional light microscopy, electron microscopy (6,7,8,131,139,187,245,387,504,520,640,704,918,963,987,1017,1065) and in tissue culture (10,11,327,613,739,848,851,947,1129).

The normal pigment epithelium forms a monolayer of hexagonal cells which appear cuboidal in vertical sections (189,743). There are 4 to 6 million cells per eye (1073,1136,1255) ranging in size from 3 to 180μ in diameter (582) with a mean diameter of 14μ at the posterior pole (1135). Less regularity of arrangement is seen peripherally with more pleomorphism (1135). The nucleus lies towards the base of the cell (Fig I 6). Frequently, binucleate cells with sometimes one or two nucleoli (1255) are seen in human tissues (1135) but

Figure 1a

Electron micrograph of retinal pigment epithelium (RPE). Horseradish peroxidase was injected into a monkey prior to sacrifice. The reaction product of the histochemical reaction is electron dense and fills the extracellular space, outlining the basal infoldings and lateral cytoplasmic membranes (large arrowheads) showing the limit of the outer part of the blood retinal barrier. The apical process are seen ensheathing photoreceptor outer segments (OS). Reaction product also collects in Bruch's membrane (BM). The basal position of the RPE nucleus is evident. Various types of pigment granules are present, including melanin (m), lipofusion (l) and melanolipofusin (ml) as well as immature melanin granules (i) and phagosomes (p) (x 7,000).

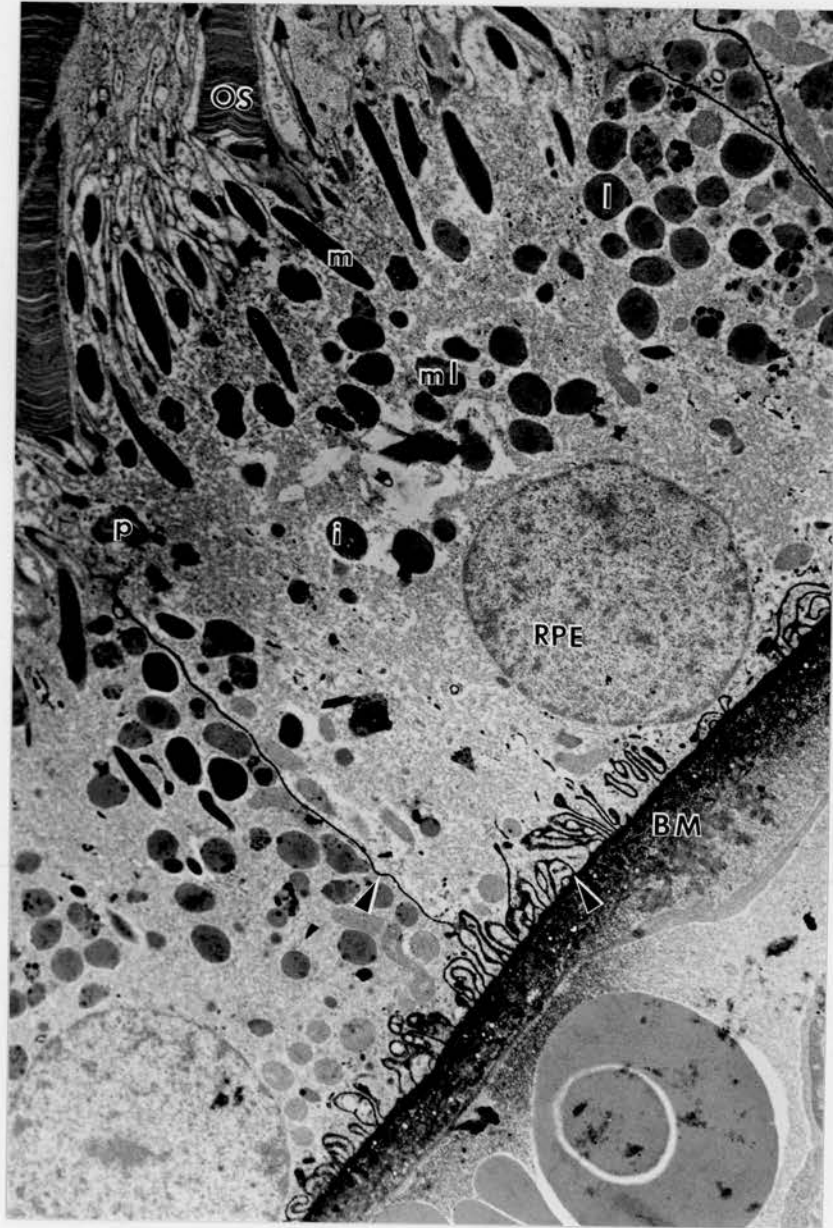


Figure 1 6

mitotic figures are not seen in the normal (8781185). The cytoplasm contains numerous organelles including mitochondria located towards the base of the cell, abundant smooth and rough endoplasmic reticulum, free ribosomes and liposomes (1255). Coated vesicles are frequently present in association with the endoplasmic reticulum and with Golgi apparatus (837) which is prominent (387,520). Microperoxisomes, membrane bound structures of 0.15 to 0.3 μ in diameter, are distributed throughout the retinal pigment epithelial cell cytoplasm, but more abundantly basally and adjacent to the lateral walls (963). These are topographically associated with lipid droplets, lipofuscin granules (865), and mitochondria (703). Autophagic granules have also been identified (951,1241). Different types of pigment granules have been described in detail (300,303,582,1086). Melanin and lipofuscin are both present within the retinal pigment epithelium, the former in granules towards the apex of the cell and the latter in the central and basal parts (300,303,304). The melanin granules are large and elliptical or circular, and are 1 to 3 μ in diameter (1255). Melanosomes at different stages of melanisation are seen in adult retinal pigment epithelium. Lipofuscin granules are less electron dense than melanin granules. Lipofuscin accumulates with age and is not present in the retinal pigment epithelium of infants (300). Granules containing melanin and lipofuscin also occur. They are termed melanolipofuscin granules (300) and are seen in the perinuclear area. The amount of melanin per cell is greatest in the macular area but decreases from the

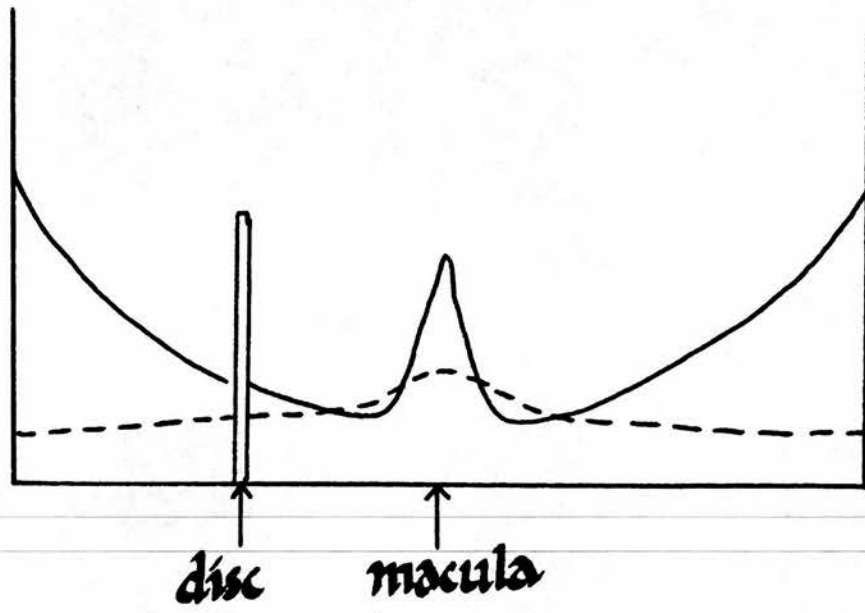
periphery to the posterior pole, while the reverse is true of lipofuscin (1184) (Fig I 7).

In the apical portion of the retinal pigment epithelial cells are phagosomes containing lamellar inclusions of photoreceptor membrane at different stages of degradation (1065). These were first described in animals by Porter and Yamada (928) and later by Dowling and Gibbons (245), and in the human by Bairati and Orzalesi (52). There are fewer phagosomes in peripheral retinal pigment epithelial cells than in those in the macular area (572).

The cytoplasmic membrane is thrown into extensive basal infoldings and long apical processes (7,8,504,520,556,690,696,740,878,987,1017,1217) which surround the outer segments of the photoreceptors completely (1065). In association with the basal infoldings is a system of intracytoplasmic tubules (640) which increase the surface area at the base of the cell substantially. The cytoplasm of the apical processes contain actin filaments (139). There are two types of apical microvilli, long (5 to 7 μ) lying between the outer segments, and short (3 to 4 μ) forming sheaths around the end of the outer segments (1073). The extrafoveal cones, unlike the foveal cones, do not reach the apex of the retinal pigment epithelial cells, but are ensheathed by the long processes which expand around the ends of the cone outer segments (131). Thirty to 45 photoreceptor outer segments are engaged by each retinal pigment epithelial cell (1238) and 30 to 40 retinal pigment epithelial cell processes surround each cone outer

Figure 17

Diagram of distribution of RPE pigments in different parts of the eye.



— melanin
- - - lipofuscin

Figure 17

segment, but many fewer surround rod outer segments (1072). There is an acid mucopolysaccharide coating the apex of the retinal pigment epithelial cells and bathing the outer segments, termed interphotoreceptor matrix (1238). This is partially synthesized by the retinal pigment epithelial cell (65,66,1106,1254) with a contribution also from the photoreceptors(871).

The lateral cytoplasmic membranes are attached to each other by junctional complexes at the junction of the apical one-third and basal two-thirds of the cell (704) (Fig.I 6). The terminal bar complexes consist of a zonula adherens adjacent to a zonula occludens more apically situated and encircling the apical part of the lateral wall of the retinal pigment epithelial cells, forming a "tight junction" (387,704,1024,1255). There are also occasional desmosomes on the lateral walls of the retinal pigment epithelial cells, but these are not continuous attachments, as are terminal bar complexes (1255).

The pigment epithelial basal lamina is a continuous membrane forming the innermost layer of Bruch's membrane.

Physiology

Choriocapillaris

The choriocapillaris has several functions largely related to supply of nutrients to the outer retina, absorption of fluid from the retina and control of the intraocular pressure by variations in choroidal blood volume. The entire choroid acts as a protective buffer for the retina, resisting deformation on eye movements (1161).

The submacular choroid has been the subject of particular attention (481,486) and is found to be remarkably similar to the choroid elsewhere. The choroid is thicker by virtue of the large number of choroidal vessels at the posterior pole, but the blood flow in the capillaries is no greater than elsewhere. In primates the flow posteriorly is 10 times greater than in the periphery (15), the larger flow measured being due to the large number of arteries aggregated in the area, and not the capillaries (15,1075).

The choroidal circulation can be visualised in vivo in lightly pigmented individuals using fluorescein angiography (20,35,222,482,545,672,1030) (Fig I 8) and also using the technique of idicyanine green angiography (82,329,330). The lobular pattern of filling demonstrated by Hayreh (484) can be exaggerated by raising the introcular pressure to slow the filling (239) and may be very irregular even in normal eyes.

Figure 1a

Early phase fluorescein angiogram showing normal filling of the lobules of the choriocapillaris.

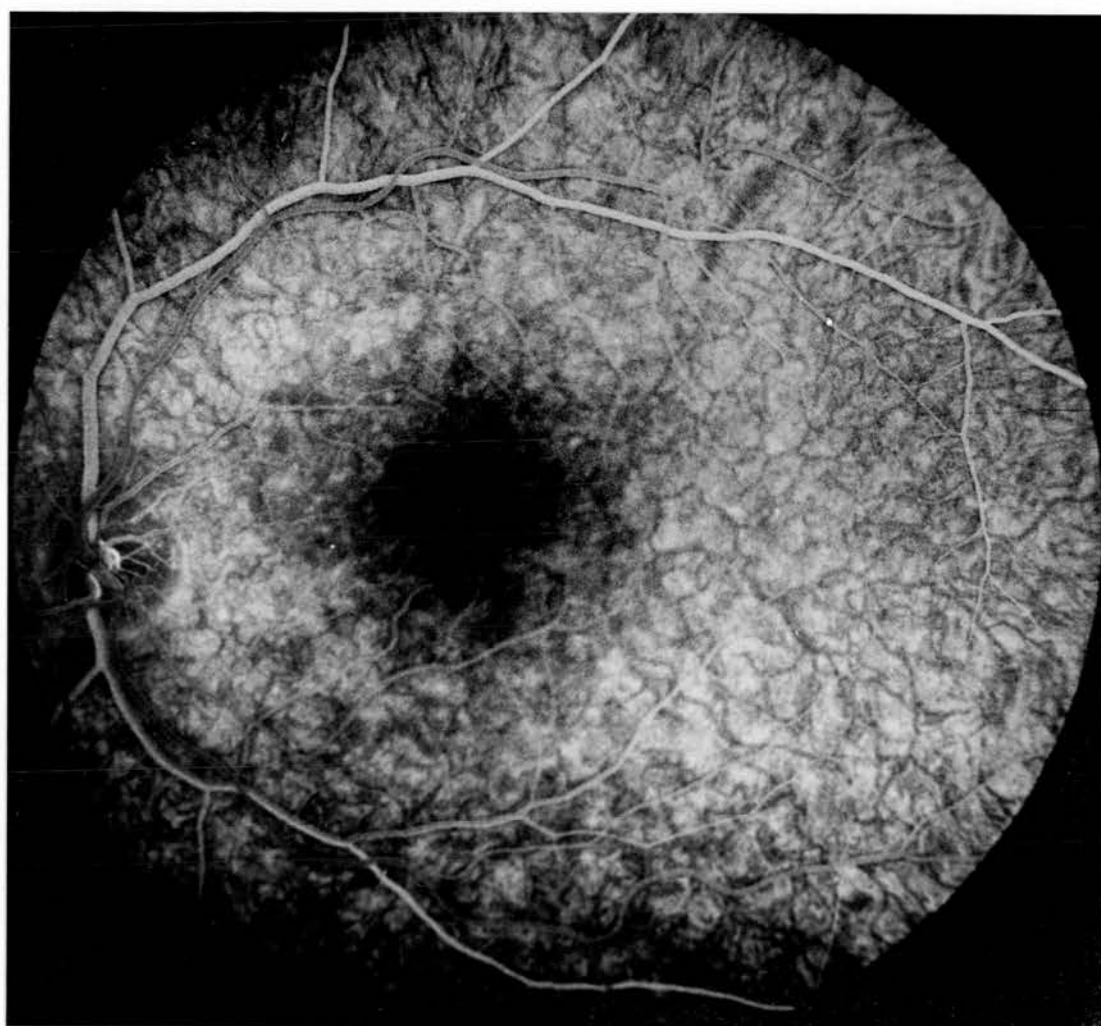


Figure 1 8

At the posterior pole the choriocapillaries fill before the retinal capillaries but in the periphery both capillary beds fill simultaneously (35).

Neonatal monkeys have a poorly pigmented RPE allowing clear visualisation of the choriocapillaris. A study of these animals confirmed Hayreh's description (480) of a functionally non anastamotic lobular pattern (908), Disagreement on this point persists, however (646,1029,1206).

Anastamotic channels between the lobules may open up under certain conditions. For example, occlusion of a ciliary vessel in the rabbit results in filling of lobules supplied by the occluded vessel, demonstrating functioning anastamosis (1212). In the same model, vortex vein occlusion results in a reversal of the blood flow, whereas raised intraocular pressure ultimately causes vascular collapse (1212). The presence of glomus cells has been offered as evidence of the functional existence of anastamoses (717) although this has never been substantiated (38).

The choroidal blood flow accounts for 85% of the ocular blood flow (400) and is 20 times the retinal blood flow (485) and 10 times the brain blood flow (1085). The flow is in excess of requirement to supply oxygen to the tissues (186), since the venous oxygen tension is 95% or more of the arterial tension. In one study only 2% of the oxygen was extracted from choroidal blood, compared

with 35% in the retina (1116). In addition only 2% of the blood glucose was extracted, compared with 12% in the retina. It is suggested that the large blood flow allows the choroid to act as a heat sink for the dissipation of heat absorbed by the retinal pigment epithelium (890). It has been observed that if choroidal blood flow is impaired, the retinal temperature rises (890). Choroidal blood flow can be measured by a number of techniques including the Krypton 85 washout method (368) reflective densitometry (425,1119) and microsphere injection (1184C).

In rabbits, the average flow of blood through the choroid is of the order of $80 \mu\text{l}/\text{sec}/\text{cm}^2$ of choroid (368,1119). Similar figures are reported for other species (14,16)

Infusion of microspheres shows that although the capillaries have a large diameter (20-40 μ) local constrictions limit intraluminal flow (14,77).

The choroidal blood flow is modified by blood gas tensions, and in cats falls in response to 100% oxygen, and rises in response to 10% carbon dioxide (367), but the response is greater with altered carbon dioxide levels than oxygen (75,1120) comparable to the pattern of blood flow control in the brain, and in contrast to retinal blood flow, which is most susceptible to altered oxygen tension.

Theoretically, the arrangement of the choroidal arterioles which end abruptly in a large calibre capillary

bed, would be susceptible to damage because of the large pressure drop across the bed unless the flow is under neural control (930). The arteriolar supply is under sympathetic control (73) protecting the choriocapillaris from this effect.

The choriocapillaris responds to a number of vasoconstrictors, including adrenalin, phenylephrine, and histamine (167,1162,1211) and sympathetic stimulation (443,1184D) although the vasoconstrictive response is less intense than that demonstrated by other capillary beds (1161). The control of the flow through the choriocapillaris is also modified by the arteriolar supply (1161) and intraocular pressure (72). Vasoconstriction causes an increase in choroidal blood flow (366).

There is a rich nerve supply in the choroid (678,1161,1203). Both alpha (73) and beta(122) adrenergic receptors have been identified pharmacologically.

However, in the normal state there is sympathetic tone (1120,1184) controlling the total blood flow to the eye, sympathectomy causing an increase in ocular blood flow while presumably having the opposite affect on the choroid. Experimentally induced hypertension with sympathectomy results in leakage of fluorescein from the choroid into the retina (275).

The choriocapillary bed is unusual in that in contrast to other capillary beds, in which the blood flow is controlled to meet metabolic demands, the blood flow has a bearing on the production of intraocular fluid.

Transudation of fluid from the choriocapillaris exceeds resorption and the excess fluid must contribute to the intraocular fluids (161).

The choriocapillaries are permeable and small molecules such as horseradish peroxidase (30 \AA) cross the endothelium, whereas larger molecules, such as catalase (52 \AA) leak more slowly (920) and ferritin (110 \AA) is largely restricted (279,789,919). Larger molecules such as thorotrast may leak out of the capillaries through the intercellular gaps rather than across the fenestrae (887,986) as in other areas of the body (541,886). The permeability to small molecules is very high (1114,1115). More than 50% of the molecules of the size of glucose and amino acids pass through the capillary wall on one passage, and the glucose concentration in the intersitital fluid underlying Bruch's membrane is 95% that of plasma. Much of the fluid which leaves the choriocapillaris is not resorbed, there being no significant colloid osmotic pressure gradient but leaves the extracellular space transsclerally, under hydrostatic pressure (76). This route accounts for the clinical observation of uveal effusion, or accumulation of extracellular fluid in the choroid when the intraocular pressure falls, and also reabsorption of subretinal fluid after surgery with the siting of a plomb, raising the intraocular pressure (Fig. I 9) It has been demonstrated under pathological

conditions that the diameter of the fenestrae alters, correlating with increased permeability (473). In general physiology, the fenestrations in a fenestrated capillary bed are known to correlate directly with capillary permeability (328), while in non-fenestrated capillaris, pinocytotic vesicles represent the mode of transport of material across the capillary wall (740). Horseradish peroxidase has been shown to be transported across capillary endothelial cells of the choroid in vesicles (383) as well as across fenestrations (920).

Pericytes are thought to control the local capillary blood flow (517,989,1199). In other parts of the body, they form an incomplete layer around precapillaries, capillaries and postcapillary venules (363,1252), and possibly regulate permeability (546).

The permeability is comparable to or greater than glomerular capillary permeability (812) being 5 times the permeability of the glomerular capillaries and 10-30 times that of muscle to plasma protein, and relatively higher for smaller molecules (78). Recent studies of permeability in the rabbit (74) indicate however, that the flux of albumin out of the vessel is less than elsewhere (74,78,917). The permeability to gamma globulin is 50% that of albumin (74).

Since retinol is transported in the blood as a protein complex of similar size to albumin, and leaves the choriocapillaris to enter the retina, this point needs to be clarified.

The nutrient supply to the retina largely arrives via the choriocapillaris (69,812), while the retinal circulation has largely a respiratory function (812), contrary to the conventional understanding that the retinal circulation serves the nutrient and respiratory demands of the inner retina, and the choriocapillaris the demands of the outer retina (672).

There is an intimate relationship between the choriocapillaris and the retinal pigment epithelium. In retinitis pigmentosa, when the retinal pigment epithelium is absent, there is often choriocapillary atrophy. Similarly, if the retinal pigment epithelium is destroyed by the systemic administration of sodium iodate the choriocapillaris atrophies (498). It has been suggested that the retinal pigment epithelium controls the choriocapillaris and that a vascular modulation factor which controls the pattern of choriocapillary fenestrations is absent if the retinal pigment epithelium is destroyed, resulting in a loss of fenestrations or occlusions of the choriocapillaris (644,737). The RPE is also thought to inhibit choriocapillary proliferation (421).

Bruch's membrane

Bruch's membrane is freely permeable to water (823,824) but is known to have a filtering function (387,928,969,1090,1100,1159,1190). Early permeability experiments indicated that the maximum size of molecules

Figure I₉

Diagrammatic representation of transendothelial fluid fluxes in the choriocapillaris.

Figure I₁₀

Diagram taken from the work of Young, illustrating rod disc shedding. Radioactive amino acids were injected into frogs, and by autoradiography, A, the incorporation into photoreceptor discs, B, the passage of the discs down the outer segments, C, disc shedding and D, RPE phagocytosis were observed.

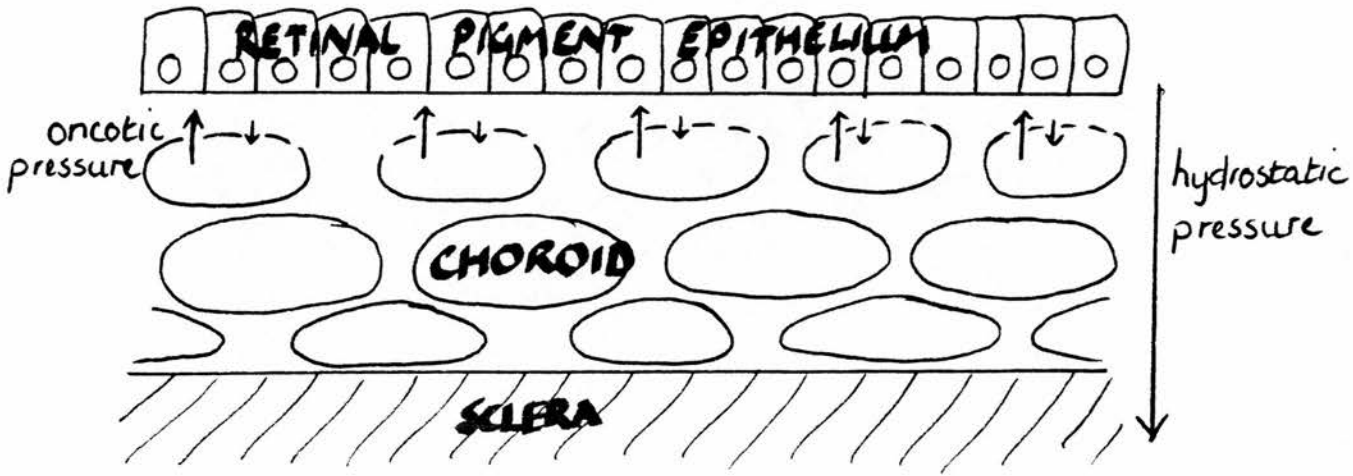


Figure 19

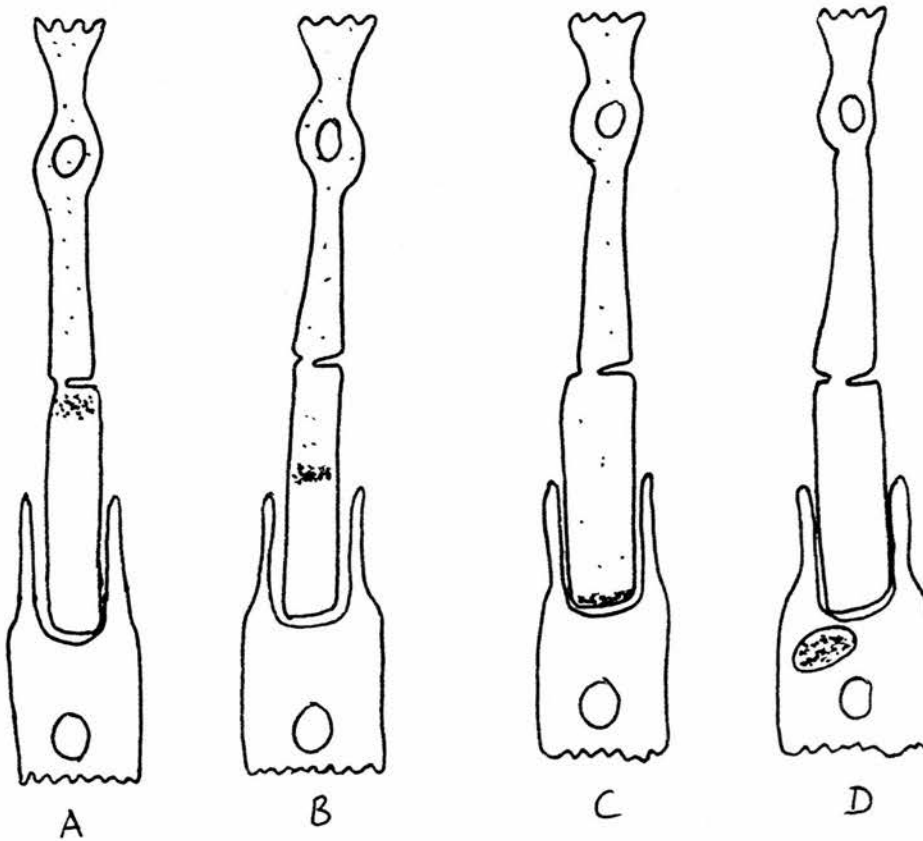


Figure 1 10

which can transverse the membrane is 150,000 MW (722). More recently, the permeability has been studied in relation to the charge of the membrane. Anionic sites have been identified (921) in a regular lattice pattern in both sides of the membrane (281) forming a charge barrier for anionic molecules. The anionic sites are formed by chondroitin and heparan sulphates (922). The charge on the Bruch's membrane is similar to the charge on the glomerular basement membrane (162). The comparison of Bruch's membrane to the glomerular basement membrane will be elaborated later, but has already been made by Nakaizumi (834) and by Leeson and Leeson (692). One major difference between the two membranes is the presence of the collagenous and elastic layers of Bruch's membrane. However it has been suggested (834) that the open porous arrangement of these layers allows free transfer of fluid, although the more complex barrier allows much more exchange of metabolites within Bruch's membrane than the simple arrangement in the glomerulus where the filtration barrier is composed of basement membrane only (504). No details of the metabolic exchange within Bruch's membrane are known.

An additional function which Bruch's membrane is said to serve is that of a stable base for the retina (431).

Although we think of Bruch's membrane as an inert structure, it is constantly being turned over in a similar way to other extracellular materials. For example, the collagens of the glomerular basement membrane have a half life of 100 days (2,933).

Retinal Pigment Epithelium

The retinal pigment epithelium is a highly metabolically active tissue, consuming oxygen at a higher rate than other tissues (192), and having a high oxidative enzyme activity (470). In conjunction with the photoreceptors, it accounts for two-thirds of the total oxygen consumption of the retina (1184E). A large part of the metabolic activity of the pigment epithelial cell is devoted to phagocytosis of photoreceptor outer segments. This subject has been comprehensively reviewed (97,100,683,1242). Phagocytosis in the retinal pigment epithelium was first recognized in 1954 by Karli (591,592) and later by Cohen (188). Dowling and Gibbons (244) had shown that in vitamin A deficiency, there is a loss of phagosomes, which were at that time termed myeloid bodies, in the retinal pigment epithelium, which return after vitamin A administration. The hypothesis of photoreceptor disc shedding and pigment epithelial phagocytosis was proposed by Bairati and Orzalesi (52) and was elegantly proven by Young using autoradiographic techniques (1236,1237). He showed that, in the frog, following the injection of tritiated leucine and phenylalanine to label newly synthesized protein in photoreceptor discs, the labelled protein moved down the outer segments and was ultimately shed, and then engulfed by the pigment epithelial cells (Fig. I 10). Young and Bok coined the term phagosome for the engulfed photoreceptor membrane material in the pigment epithelial cell and noted that they migrated from the apex to the

base of the cell (1240,1245). The process of outer segment phagocytosis has been studied morphologically in the human (52,522,1065,1238) and in other species (382,527,755), and in cell culture of both human (108,1129) and other species (263,476,848,988). Test materials for phagocytosis, for example, polystyrene spheres (108,382,476,527), liposomes (263) or bacteria (476,527) have been introduced into the system, either into the culture medium (108,263,476) or into the subretinal space (382,527). It appears that the nature of the phospholipid membrane presented to the retinal pigment epithelium cell influences the rate of phagocytosis (263) with selective phagocytosis of other material present (527). When microspheres of different sizes are presented, the large spheres are engulfed totally, while the smaller ones are aggregated in a glycosaminoglycan matrix before being phagocytosed. However, if a large load of phagocytic material is presented, histocytes which are not selective of the material phagocytosed invade the subretinal space (382). Further evidence confirming the hypothesis is gained from the observations that following experimental retinal detachment, there was a loss of phagosomes which returned following reattachment (655,656). Light damage produces a remarkable increase in the number of phagosomes (458,665), as does ischaemia (572).

The size of the metabolic load presented to the pigment epithelial cell is astounding. Calculations (1239) show that in the rhesus monkey, each rod assembles on the order of 80 to 90 discs per day, and 25 to 45 rods insert

into the apical processes of each retinal pigment epithelial cell. At the fovea, 2,000 disc per day are phagocytosed by each retinal pigment epithelial cell, and at the periphery, 4,000 discs per day. This represents the phagocytosis of up to $9,000\mu^2$ of double membrane per cell per day. On top of these astonishing figures, a large reserve capacity has been demonstrated (458,661,665,750).

As digestion occurs, the phagosomes, now phagolysosomes, break down, leaving the residue in the retinal pigment epithelial cell cytoplasm in the form of lipofuscin granules (305,755). The membrane discs are easily recognisable at the apex of the retinal pigment epithelial cell, but as they migrate towards the base of the cell, digestion takes place, and the membranes become more closely packed and electron dense. Apart from the lipofuscin accumulation, oil droplets are seen in the cytoplasm, and are suggested to represent a portion of lipid from the outer segment membrane, the other portions possibly being rapidly recycled (53). The microperoxisomes may have a function related to lipid turnover (963), possibly in association with the coated vesicles which are considered to have a function in transport of metabolites within the cell (1135). Some particulate matter, and presumably also soluble material, is extruded from the cell through the lateral walls to be passed into the subretinal pigment epithelial space (978). A cytoskeletal contractile mechanism for this process has been identified (978).

Although the fate of the phagocytosed material is not fully known, the products of digestion are somehow discharged into the subretinal pigment epithelial space from where they traverse Bruch's membrane, to be carried away by the choriocapillaris. Hogan (520) has suggested that the breakdown products are packaged in vesicles some of which collect in Bruch's membrane and accumulate with age. Defects in this complex system must contribute to various disease states (299).

Apart from the phagocytosis of outer segment discs, the retinal pigment epithelium is active in phagocytosis as a defense against injury. In many pathological circumstances, they are seen to be phagocytic, and will be discussed in detail in the section on pathology.

Interest in the retinal pigment epithelial cell function is also directed towards its role in the maintenance of the blood-retina barrier and in the maintenance of neuroretinal apposition. The structure of the retinal pigment epithelial cell indicates its participation in fluid transport. When ultrastructural studies of tissues were in their early stages, at a time when electron microscopy first became widely available, basal infoldings were described in cells which are known to transport a large fluid volume across them. The first such cell was the epithelial cell of the proximal tubule of the kidney, and later epithelial cells of the submaxillary gland, choroid plexus and ciliary body were found to have a similar structure (898). The basal

infoldings of the retinal pigment epithelial cell probably represent a similar large fluid load.

It has long been recognised that in the days following retinal detachment surgery, subretinal fluid gradually disappears (710). The retinal pigment epithelium pumps fluid from the subretinal space towards the choriocapillaris, maintaining neuroretinal apposition in the normal state (214,347,803,1248). Various ion pumps have been characterised (84,238,341,347,608,666,685,808,1070,1071). These pumps control the quantity of fluid, creating a pressure difference across the retina (770), as well as controlling the ionic composition of the subretinal fluid (1019). There is a sodium-potassium pump at the apex of the cell and a bicarbonate pump at the base (537). Sodium and calcium ions are transported across the apical membrane in the choroid to retinal direction and chloride ions are transported across the basal membrane from retina to choroid (85). The exact mechanism by which these pumps are coupled to fluid flow has not yet been determined. In the bull frog in the absence of hydrostatic pressure the net flow of fluid across the RPE is from the retina to the choroid, at a rate of 4.8 $\mu\text{l}/\text{cm}^2\text{hr}$, largely under the control of cAMP (804). Recently, Negi and Marmor (842,843) have further characterised the flow of fluid from the subretinal space to the choriocapillaris and suggested that 70% of the transport mechanism is active, mediated by the retinal pigment epithelium, and 30% pressure mediated by the oncotic pressure in the choriocapillaris (Fig. 111). The

Figure I₁₁

Diagram summarising the fluxes of fluid and solute movement controlled by the RPE.

Figure I₁₂

Diagram showing the sites of maintenance of the posterior blood eye barrier. The outer barrier is formed by the tight junctions between RPE cells, and the inner by interendothelial cell tight junctions in the retinal circulation.

FLUID

active transport

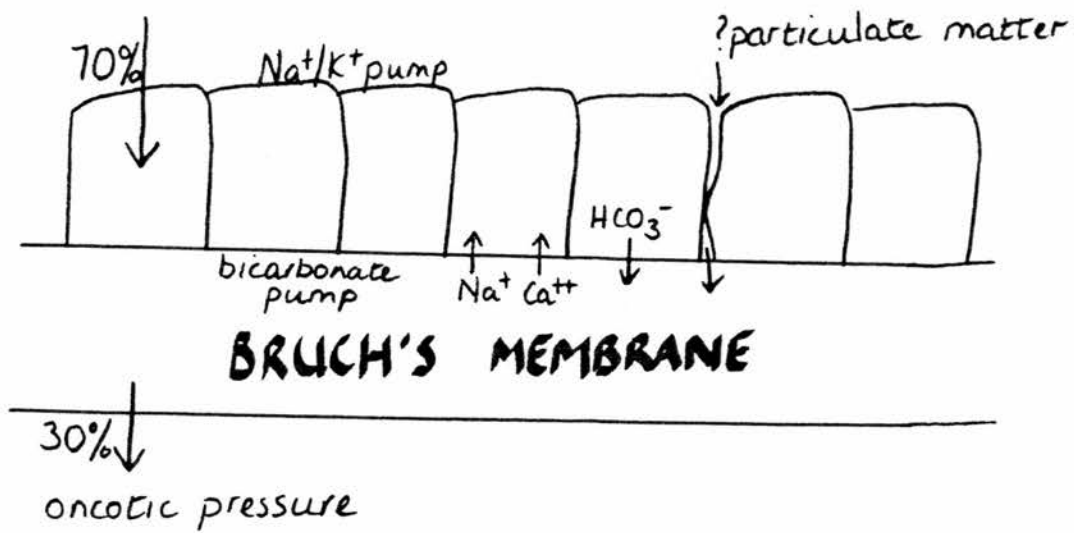


Figure 1 11

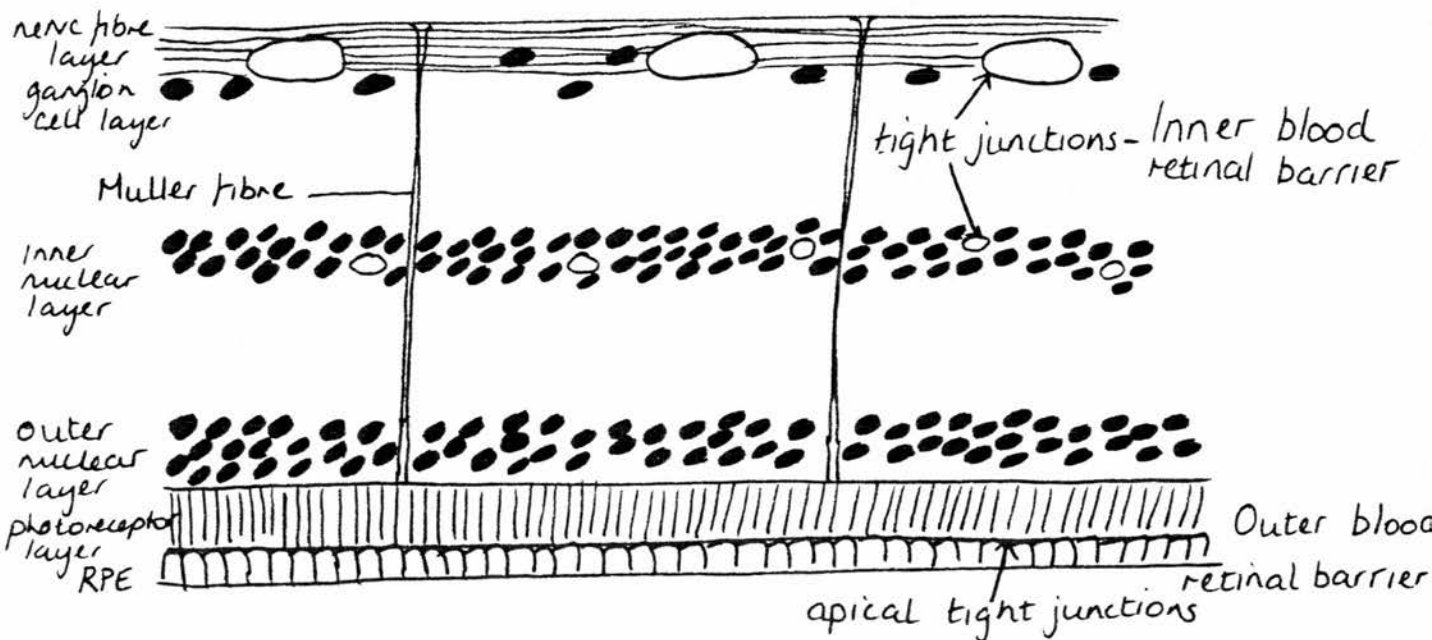


Figure 1 12



transport mechanisms can be manipulated experimentally, and when blocked by benzyl penicillin (214) or ouabain (1070,1246) for example, result in retinal detachment. Acetazolamide enhances the pumping function, sometimes to therapeutic advantage (749,1144,1145). If the retinal pigment epithelium is diffusely damaged, the resorption of subretinal fluid is compromised (840). However, if it is resected, retinal detachment does not ensue (344). In the latter case, it is likely that the scarring process following trauma replaces the normal retinal adhesive properties of the retinal pigment epithelium (1247,1250). Following retinal detachment, the retinal pigment epithelium and the photoreceptors are reapposed, but the complex intracellular relationship is not returned to normal, even after a short period of detachment (22).

The maintenance of the blood-retinal barrier by the retinal pigment epithelium is of sufficient importance in the discussion of the pathophysiology of chorioretinal disease to merit more detailed discussion. The blood eye barrier is comparable both in structure and function of the blood brain barrier (242,941). In the posterior segment of the eye, the barrier has two components, an inner and an outer (190,211,212,213,942). The inner barrier is maintained by the endothelial cells of the retinal vasculature and the outer by the tight junctions between adjacent retinal pigment epithelial cells (Fig I 6,12). The barriers can be demonstrated histopathologically in experimental animals using morphological tracers, such as fluorescein (444,783), horseradish peroxidase (154,642,1022,1032,1113),

microperoxidase (282,1047), silver (1190) or tritiated water (881). In vivo, the blood-retina barrier is conventionally assessed using fluorescein angiography (202,1024) but more recently, vitreous fluorophotometry has been developed as a research tool for quantifying defects in the barrier (164,977).

The blood eye barrier limits the passage of all fluids, metabolites and drugs with only dissolved gases having free access from the circulation to the tissues. All other substances gain access to the normal retina by facilitated or active transport across the barrier (85). Morphological tracer techniques have also been extensively employed in demonstrating pathological breakdown of the blood-retinal barrier, for example, after photocoagulation (911,912,913) after iodate poisoning (27,219) and after cryotherapy (215).

It is logical to discuss at this point lesions thought to be caused by defects in the outer blood retinal barrier. We are no longer concerned with the inner blood-retinal barrier. Hyperosmolality of the blood has been shown in experimental animals to disrupt the blood-retinal barrier (679) contributing to the controversial clinical issue of whether or not a defect in the blood-retinal barrier, either inner or outer both, is the primary defect in diabetic retinopathy (272).

Tso has reviewed the morphologic correlates of blood-retinal barrier disruption both at the level of the

retinal capillaries and at the level of the retinal pigment epithelium (1122).

The retinal pigment epithelial component of the barrier may be broken by disruption of the cells, as occurs after occlusion of the short posterior ciliary artery (1122,1124) or after severe photic injury (1174), or the cells may undergo necrosis as a result of photic injury, ischaemia (1122), or the intercellular junctions may be broken down in proliferation of retinal pigment epithelium over choroidal tumours (1172). Finally, in some eyes, the retinal pigment epithelial cells appear to fail to function without being frankly disrupted or necrotic. This decompensated state is identified in experimental animals following lens extraction (1139) commotio retinae (133) or hypotony (1122,1134,1138).

Clinically, a persistent abnormality of the blood-retinal barrier has been identified in aphakia (813) with a reduction of movement of fluid out of the eye up to two years following surgery (816). The long term defect is also identified after laser coagulation (346). The functional significance of this observation is obscure (816).

In monkeys, a leakage of the outer blood-retinal barrier has been shown to persist for some years after disruption by photic injury, but the neuroretina appears to tolerate the leakage, at least morphologically, in that the photoreceptors are histologically intact (1122). However, if the retinal pigment epithelium persistently

leaks more profusely, eventually retinal oedema and decompensation of the photoreceptors will ensue (115,497,1123,1124,1125,1126,1133). An example of this is seen in retinitis pigmentosa in which the RPE is atrophic and the outer blood retinal barrier is therefore defective. Cystoid macular oedema occurs in some of these patients (317,548,849).

Commonly retinal pigment epithelial failure is considered to be age-related or degenerative . Machemer has suggested that cystoid spaces develop because of disruption of the metabolic interaction between the pigment epithelium and neurosensory retina, since he could produce retinal oedema experimentally by creating retinal detachment (725).

Inflammatory lesions of the choroid and RPE, for example, toxoplasma chorioretinitis, Harada's syndrome and histoplasma chorioretinitis cause defects in the outer blood retinal barrier (227), and may be associated with retinal oedema.

In central serous retinopathy, fluid accumulated between the retinal pigment epithelium and the neurosensory retina, does not cause oedema of the retina. This disease has long been considered to represent a defect of the blood-retinal barrier (227,1024), but the net flux of fluid in the absence of the retinal pigment epithelium is outwards, due to the osmotic pressure in the choriocapillaris (843). A new hypothesis has been proposed for the pathogenesis of this disease, which is

that an unidentified insult causes the retinal pigment epithelium to secrete rather than to absorb fluid (841,1063,1069).

In the atrophic form of age-related macular degeneration, when parts of Bruch's membrane are denuded of retinal pigment epithelium, the blood-retinal barrier is restored by Muller cells which may elaborate basement membrane material (1184B).

Recently, it has been suggested that the breakdown of the outer blood-retinal barrier allows chemostatic and mitogenic substances normally present in the retinal pigment epithelium to gain access to the vitreous, promoting neovascular proliferation in the vitreous cavity (148).

Pigment epithelial cells influence neighboring structures in a multitude of other ways. For example, they synthesize the glycosaminoglycans which bathe the photoreceptor outer segments (1254) and at least some components of the glycoproteins and collagens of Bruch's membrane (151,273). As has already been mentioned they may polarise the choriocapillary endothelial cells (737) and induce choriocapillary differentiation (641,643,644). However, it is also postulated that retinal pigment epithelial cells normally inhibit vascular proliferation in the choroid, and are attracted chemotactically by proliferating endothelial cells (149), possibly limiting the extent of neovascularization in pathological circumstances. A statement that RPE cells stimulate

neovascularisation (359) is probably erroneous overshadowed by the weight of evidence to the contrary.

The role of the retinal pigment epithelium in the complex but ill-understood process of phototransduction is central (853). The transport of many important metabolites from the choriocapillaris to the photoreceptors is controlled by the retinal pigment epithelium. For example, binding proteins for vitamin A are present in retinal pigment epithelial cells (99) as well as in interphotoreceptor matrix (3,668,714) and Muller cells (134).

The visual pigment, rhodopsin, in its active form is a combination of opsin and 11-cis retinene, an isomer of a vitamin A aldehyde. During the phototransduction event, the retinene is isomerised to all-trans and splits apart from the opsin. The process by which the rhodopsin is regenerated by reisomerisation to the 11-cis form, is thought to occur in the retinal pigment epithelium (243). There are specific receptor sites in the basal infolded cytoplasmic membrane (669) allowing active accumulation of metabolites in the RPE. Intracellular proteins transport metabolites to the apex of the cell for transfer to photoreceptors (752).

The retinal pigment epithelium absorbs 90% of the light entering the eye (414). The melanin granules protect the ocular structures from the toxic effects of irradiation and prevent light scattering (130,303,540,971).

Albinism, in which melanin is lacking, is associated with poor macular function, although this is not all attributable to the lack of melanin in the retinal pigment epithelium, since there are also anomalies of higher visual pathways (207).

3. Pathology

In view of our poor understanding of the pathophysiology of the majority of disease affecting the chorioretinal structure, I have chosen to review the pathology on an anatomical basis, rather than to discuss diseases following an aetiological classification. Inevitably, there is a cross-over requiring some repetition.

Choriocapillaris

Although I am largely concerned with considerations of the pathology of the capillary layers, the pathology in most instances affects all three layers. Where the involvement of the capillary layer is absent or inconsequential, disease processes have been included cursorily, simply for completeness.

The classification to be followed is shown in table I 1.

Table II Pathology of the Choroid

(i) Congenital

Coloboma

Pigmentation defects

Neurofibromatosis

Harmatoma

Choristoma

(ii) Acquired

Circulatory

Inflammatory

Dystrophies

Tumours

Trauma

Neovascularisation

(iii) Aging and degeneration

(i) Congenital

Coloboma of the choroid is classified into typical when it can be related to failure of the foetal fissure to close, and atypical when the site of the defect cannot be related to the foetal fissure (882).

Pigmentation. Hypopigmentation of the choroid occurs in association with hypopigmentation of the retinal pigment epithelium in some forms of oculocutaneous albinism (447). Hyperpigmentation relates to an increased number of melanocytes in the choroid, and is termed melanosis oculi, or nevus of Ota if associated with skin hyperpigmentation on the face (448).

Neurofibromatosis may affect the entire uveal tract when diffuse neurofibromata (335) and ovoid bodies, consisting of Schwann cells wound around each other (1204), are found.

Harmatoma. Localised haemangiomas may be found in the choroid as an isolated finding, but diffuse lesions are associated with Sturge-Weber syndrome (1026,1192).

Choristoma. Bone may be present in the choroid as a choristomatous malformation (405,1186A).

(ii) AcquiredCirculatory disturbance

The circulation through the choriocapillaris is disturbed in vascular disease, principally degenerative arteriosclerosis and hypertension. With age, the choroidal blood vessel walls are thickened (41,372,471), with variation in the thickness of the vessel wall. In association with benign hypertension, intimal thickening and endothelial proliferation are present in choroidal vessels of all sizes at the posterior pole, and are more advanced than the changes in retinal vessels of the same patients (41,471,1150). In accelerated hypertension, hyalinisation, fibrinoid necrosis of the choroidal arteriolar walls and trombosis are seen (193,372,413,471,1150), with occlusion of the choriocapillaris (41,471,1150). The spectrum of vascular changes in the choroid in benign and accelerated hypertension is similar to that seen in the kidney (41). Thrombi and fibrinoid necrosis of the vessel walls of the choriocapillaris result in acute occlusion with infarction of the pigment epithelium. The acute effect is serous retinal detachment, or later, the clinical identification of Elschnig's spots. These are small yellow spots with central pigmentation (624) which histopathologically are identified as patches of reactive retinal pigment epithelium overlying foci of obliteration of the choriocapillaris (135,822).

Hypertensive choroidopathy has been studied in a model in rhesus monkeys (235,614). In the acute phase, there is

arteriolar constriction with focal necrosis of the choriocapillaris and retinal pigment epithelium and serous retinal detachment. Later occlusion of the arterioles and capillaries as seen in human cases occurs. Subsequently, there is a reparative phase in which subretinal fluid is resorbed, leaving diffuse patchy depigmentation of the retinal pigment epithelium and arteriolisation of the choriocapillaris (822). The choroidal circulation shows more severe disturbance than the retinal circulation which has better structural support, and is protected by autoregulation (235).

Obstruction of the flow in the choriocapillaris occurs in normotensive patients and also in association with sickle cell disease, polyarteritis (494) giant cell arteritis (1067,1175A) and so-called choroidal spasm (19). Coat's (179) described a patient with posterior ciliary artery occlusion with infarction of the choroid, retinal pigment epithelium and full thickness of the retina. Hepburn (501) described a pigmentary degeneration following occlusion of a choroidal artery, and Maumenee (768) showed loss of the choriocapillaris in patients with pseudoretinitis pigmentosa. RPE mottling is a common end result of acute or chronic choriocapillary insufficiency. In the cases of presumed acute obstruction of the choroidal circulation, clinical findings have been described with a geographic patch of retinal oedema acutely, and pigment mottling after recovery (19,345,1140). Histopathological study of one eye showed absence of the outer retina and retinal pigment epithelial degeneration (345).

In a series of experiments in monkeys, Hayreh demonstrated the effects of ciliary arterial (479,489,490) and vortex venous occlusion (491). Vortex vein occlusion causes a syndrome similar to the clinically recognised symptoms of anterior ischaemic optic neuropathy, with the appearance of an intense inflammatory exudate. Clinically white lesions in the fundus which develop into depigmented scars over the ensuing 2 to 3 weeks follow posterior ciliary artery occlusion. Histopathologically (490) the choriocapillaris and Bruch's membrane are unremarkable, but disruption of the RPE showing a wide range of severity was observed, being disorganised acutely and later proliferating to fill the defect. Recovery occurs by shunting of blood (277)

Microvascular obstruction occurs in disseminated intravascular coagulation. Histopathological features are retinal detachment, choroidal haemorrhage and RPE vacuolation (183). Focal acute occlusion of the choriocapillaries is a histopathological feature of laser photocoagulation (29).

Chronic ophthalmic ischaemia results in loss of visual function and neovascularisation of the retina. The relative contributions of the retinal and choroidal circulatory failure to functional loss in this situation is not known (129).

In a series of patients with simultaneous acute obstruction of the retinal and choroidal circulations, it has been noted that the visual defect is more profound and the intensity of the retinal opacification greater than in patients with retinal arterial occlusion (120), indicating that the choroidal circulation contributes to retinal function. In experimental animals with retinal circulatory failure, the choriocapillaris is capable of maintaining some retinal function by diffusion of nutrients (672).

The circulation to the choroid is restricted in carotid artery disease (1079) but the ocular effects are much more severe and widespread than those caused by a disturbance of the posterior ciliary circulation. Choroidal circulatory failure has also been recently observed as a complication of closed vitrectomy (411) in which the intraocular pressure may be raised sufficiently to cause a failure of choroidal blood flow, which returns after surgery. The retina becomes opaque and after recovery, there is residual pigment mottling. This condition has been reproduced in an experimental model (888). An opportunity arose to examine histopathologically the eyes of a patient with a selective occlusion of the choriocapillaris while the retinal circulation was totally spared in a case of primary amyloidosis (1130). In this case, the retinal pigment epithelium and full retinal thickness remained intact. It is possible that what appeared morphologically to be an obstruction of the choriocapillaris was functionally patent.

Acute multifocal placoid pigment epitheliopathy may be due to vasculitis affecting precapillary arterioles in the choriocapillaris, causing an overlying pigment epitheliopathy in the distribution of the choriocapillary lobules (227,230).

The role of the choriocapillaris in the pathophysiology of macular oedema is discussed in the section on defects in the blood retinal barrier in the review of retinal pigment epithelial physiology.

Recently, more attention has been paid to the choroid in diabetes (252). In the retinal vessels in diabetic retinopathy, the hallmarks are thickening of basement membranes and loss of pericytes (184,630,1224,1225) and the study of diabetic retinopathy has overshadowed interest in the choroid. Isolated observations in histopathological specimens have been made, but could not be interpreted as diabetic, as opposed to aging changes or hypertensive vascular disease. Cogan and Kuwabara stated that the choroid is normal in diabetic eyes (184). However, basement membrane thickening has been noted in the choriocapillaris (289) and with accumulation of elastin (814) and Yanoff (1225) described PAS (periodic acid Schiff) positive deposits in arteriolar walls with occasional obliteration of the lumen. The lesions have recently been described in detail by Hidayat and Fine (508) who compared the nodules to those in the kidney in Kimmelsteil-Wilson disease. They suggest that these lesions are specific for diabetes, and have also observed

a neovascularization process in the choroid, which they term proliferative choroidopathy. Animal models of diabetes also show abnormalities of the choriocapillaris (94,529). There is evidence from clinical studies of a functional filling disturbance in the choriocapillaris (364).

Inflammation

Inflammation may be due to infective or noninfective agents. A detailed account with comprehensive bibliography of the infective agents known to cause choroidal inflammation has been given by Green (448). To summarise, the infective agents may be bacterial, viral, fungal or protozoan.

Any bacterium which goes through a bacteraemic phase may be lodged in the choroid and set up an inflammation. Experimentally, this has been demonstrated by introducing bacteria into the circulation of dogs and examining the eyes. There was multifocal septic choroiditis with serous retinal detachment, histopathologically seen as microabscesses in the inner choroid and subretinal pigment epithelial space (794). Important bacterial forms of choroiditis include leprosy (248), and tuberculosis, which are becoming rare in the western world (1207). Descriptions of choroidal tubercles exist in the literature (91,112), although bacilli are rarely found in these lesions. Chorioretinitis associated with syphilitic infection is characterised by a pepper and salt appearance of the fundus. Histopathologically,

there are large scarred areas with loss of outer retina and pigment epithelium, and some large vessels of the choroid. Within the scarred areas, retinal pigment epithelial cells and glial cells from the retina proliferate through defects in Bruch's membrane (92). Infection with nocardia occurs in traumatised eyes or in immunocompromised individuals and produces a necrotising chorioretinitis.

Viruses such as herpes simplex and cytomegalovirus cause a retinitis rather than a choroiditis, although herpes simplex viruses have been isolated from the choroid (448). Herpetic chorioretinitis has been modeled in rabbits (873).

Fungal infections are becoming increasingly frequent, as the size of the immunocompromised population grows, either through abuse of drugs, therapeutic immunosuppression, or the spread of AIDS. *Candida* endophthalmitis is the commonest (511) and coccidiomycosis rarer (966). Other fungal infections characterised by necrotic and granulomatous inflammation are blastomycosis (337) and spirotrichosis (175,353). In the immunocompromised, especially in drug abusers, asperigillus infection produces a suppurative choroiditis (833). Histoplasmosis is endemic in certain parts of the USA. The severe visual damage which can occur following histoplasmic infection is the end result of a self-limiting choroiditis, which occurs when the blood-borne organism lodges in the choroid (1181A). The inflammatory response may rupture Bruch's membrane, and the healing

process includes neovascularization, with its attendant severe effects on retinal function (607). The process has been studied histopathologically in the human (549,607,790,1025) and in monkey eyes with experimentally induced lesions (570,1044,1045).

Toxoplasma chorioretinitis caused by a protozoa is thought to account for 50% of the granulomatous uveitis cases (1098). The infection occurs in utero (903) and has been studied in experimental models (250,542,689,784). Macrophages are a prominent feature of the inflammatory response and monocytes from the choroidal circulation enter the subretinal space and phagocytose photoreceptor outer segments. The histopathological studies in human cases show largely a retinal necrosis, with a variable choroidal infiltrate, sometimes granulomatous (940) and sometimes nongranulomatous (850,1231). Lesion occur more frequently in the macular area than elsewhere. This is presumed to be due to the pattern of the circulation in the choroid.

Various non infective forms of choriocapillaris are recognised. In sympathetic ophthalmia, one of the diagnostic signs is said to be sparing of the choriocapillaris while the remainder of the choroidal layers are thickened by a granulomatous infiltrate (259). More recently, this classical description has been found to be at fault, with 40% of cases showing involvement of the choriocapillaris (210). Dalen Fuch's nodules are also a part of the classical description of sympathetic ophthalmia. These consist of epithelioid cells internal

to Bruch's membrane, formed in approximately one-third of cases (718). These nodules are now considered to be composed of metaplastic retinal pigment epithelial cells (336,554) rather than of mononuclear phagocytes as was previously held to be the case.

Phakoanaphylaxis is related to sympathetic ophthalmia, having similar histopathological features and being found in anything from 23%(90) to 46% (718) of cases of sympathetic ophthalmia. Vogt Koyanagi Harada syndrome also has many clinical and histopathological similarities to sympathetic ophthalmia, although some report that the inflammation in the choroid is nongranulomatous (907), while others report that it is granulomatous (448,547).

Sarcoidosis is an inflammation of unknown etiology in which 10% of cases manifest a choroiditis and 11% have chorioretinal nodules (869).

Birdshot retinopathy is a bilateral inflammatory disease in which multifocal lesions are identified at the level of the retinal pigment epithelium (982). In one case in which vitrectomy was undertaken, a biopsy showed granulomatous uveitis (271). In one case examined in detail histopathologically, the granulomatous inflammatory lesions in and under the retina were considered similar to S antigen induced disease in monkeys, suggesting an autoimmune mechanism (866).

Other chorioretinal disorders are thought to be primarily of lesions of the choroid, but no histopathological

reports exist. Examples of these are serpiginous choroiditis, in which confluent exudate and scar spread out from the disc (398), and acute multifocal posterior placoid pigment epitheliopathy, already mentioned under the heading of circulatory disturbance.

A noninflammatory cellular infiltrate is seen in the choroid in lymphoproliferative disease, particularly large cell lymphoma (9). The malignant cells form a diffuse infiltrate (1163) and tend to form masses between the retinal pigment epithelium and Bruch's membrane (809). A case of chronic granulocytic leukaemia has been reported, in which choroidal involvement was associated with an exudate in Bruch's membrane (1205).

Dystrophies

The term choroidal sclerosis encompasses a group of diseases with one clinical feature in common, namely retinal pigment epithelium atrophy, allowing direct visualisation of the choroid. These have been classified by Krill and Archer (648) according to the presence and distribution of choriocapillary atrophy in association with the retinal pigment epithelial atrophy. Scattered histopathological reports exist in the literature. Central areolar choroidal sclerosis is characterised by atrophy of the choriocapillaris with atrophy of the overlying retinal pigment epithelium and outer retina (39,158,315,534,1053). In regional choroidal atrophy, and in a case clinically resembling sectoral retinitis pigmentosa (943). Similar changes are found, but in a

different distribution (1184B). The primary defect in choroideraemia also appears to be loss of the choroid, and secondary retinal degeneration (147,775). Recently it has been proposed that the initial lesion is in the choroidal endothelial cell, which is recognised by its failure to produce basement membrane (147).

Neoplasia

The range of neoplastic lesions of the choroid are mentioned only for completeness. Nevi are often associated with nodular or diffuse deposits in Bruch's membrane (839). Leiomyomata have been reported rarely (562).

Malignant melanoma is a primary tumour of the choroid. Metastatic tumors occur frequently and have been reported in a post mortem study to occur in 12% of cases of malignant disease (89) being 68% from breast and 11% from lung.

Trauma

Choroidal tears may occur as a result of contusion injury. Histopathologically, there is absence of the choroid and retinal pigment epithelium at the site of the injury (466,600). Such injuries may be complicated by neovascularization (4,379,401,432,472,510,898,1046).

Choroidal haemorrhage and effusion with detachment may cause serious intraocular disruption.

Neovascularisation

This subject has been recently reviewed by Green and Wilson (452). The growth of new vessels from the choriocapillaris through Bruch's membrane and under the retinal pigment epithelium is the final common path for many injuries and diseases of the chorioretinal juncture and is the cause of the major part of the visual loss in affected patients. The documented disease entities in which choroidal neovascularization have been described clinically and histopathologically are tabulated by Green and Wilson (452). A simpler table is offered by Henkind (496). The lists include degenerative disorders, inflammatory diseases (602), tumors (719,1048), and post traumatic (629,1187,1213), some of which have already been covered in more detail.

Vascularisation of the peripheral subretinal pigment epithelial space is described (581), but may be a variant of normal anatomy (452,1064). Pathologically, it is associated with longstanding retinal detachment (448,581). Peripheral disciform reactions do occur, particularly in elderly hypertensive patients, but are of lesser clinical significance than macular lesions (25).

The first histopathological description of macular subretinal pigment epithelial neovascularization was made in 1928 (526). Ashton and Sorsby were the first to describe the disciform response as a scarring reaction (42), and later, Gass drew up a hypothesis which

encompasses our present understanding of disciform macular degeneration, with neovascularization being the first stage of the response, which then leads to exudation and an organised subretinal pigmented epithelial scar (393,394,401). The first detailed clinicopathological studies were reported by Sarks (993) who observed subretinal pigment epithelial neovascularization in the macular area in 20% of the eyes of elderly patients. Green and Key (450) described the histopathological findings in 176 eyes of patients with age-related macular degeneration and found neovascularization in 55% of eyes. Other authors have found sub RPE neovascularisation in 20 (993) to 80% (956) of aged eyes.

The neovascular process has been extensively studied both in experimental animals and in vitro. Rupture of Bruch's membrane is regularly observed in cases of subretinal pigment epithelial neovascularization, and was therefore considered the starting point for inducing experimental neovascularization. Norton and coworkers (863) and later Ryan (980) attempted to disrupt Bruch's membrane enzymatically with only limited success.

Photocoagulation scars more reliably produce a neovascular response in monkeys (32,33,980) but the best model to date is a combination of photocoagulation and retinal ischemia, produced by venous occlusion (32,33,34,980,981). Clinical studies of these experimental models do not reliably document the presence of vessels, some of which leak fluorescein and others not (805,806,874). The newly formed capillaries are made up

of immature endothelial tubes surrounded by pericytes and basement membrane (32,33,34). In the retina, a similar process is seen, with pericytes and endothelial cells being intimately related (386,1102). Fenestrations later appear in the vessels (805,806,874) and larger vessels mature to form arterioles and venules (32,33,34). Retinal pigment epithelial cells proliferate around the new vessels (807) possibly limiting the extent of the disruptions of the chorioretinal juncture by restricting the leakage of vessels.

More recently, the concept that Bruch's membrane must be ruptured before subretinal neovascularization can occur has been questioned (503,925). The structure of Bruch's membrane does not offer an anatomical basis for the exclusion of blood vessels, and the earlier concept may be based on a misinterpretation of observations in some cases, although clearly, there is clinical evidence that rupture of Bruch's membrane, for example, in angioid streaks and in trauma cases, is associated with subretinal pigment epithelial neovascularization. Experimentally, endothelial cells are shown to degrade types IV and V collagens if placed in a chamber with a collagen containing filter separating them from a retinal extract (586).

In vitro studies are numerous in the vascular pathological field and also in ophthalmic research. An ocular angiogenesis factor was first hypothesized over 30 years ago (43,799). Subsequently, the existence of a similar substance in tumours was proposed (114,332,333).

Tumour angiogenesis factor has been introduced into the vitreous cavity of rabbits to model neovascularization in the vitreous (321) and other similar experiments have developed from these studies, specifically directed at retinal neovascularization (297,422,894,895). Apart from the theory of the angiogenesis factor as a stimulus for vascular proliferation, other proposed stimuli are hypoxia (894,1194) and inflammation (609,626,894,1033). Some authors have interpreted the presence of inflammatory cells in Bruch's membrane in patients with age-related macular degeneration and neovascularization, as evidence of an inflammatory component in the aetiology of vascular proliferation (899,900). In vivo and in vitro studies show that macrophages induce endothelial proliferation (926).

The growth of cells in culture is influenced by the nature of the collagen on which they are grown (616,819). Endothelial cells in culture grow out as flat sheets in interstitial types of collagen and only form tubular structures on basement membrane collagens, which they synthesise themselves (559,645) or on which they may be cultured directly (728).

It is conceivable that abnormal deposits in Bruch's membrane and even basement membrane material may promote neovascularisation, particularly in degenerative conditions.

Retinal pigment epithelial cells are chemotactically attracted to endothelial cells stimulated to proliferate

by some eye derived growth factor (150) and inhibit endothelial proliferation (149). A disturbance in these interactions could also be implicated in the diseased state. However, the relationship is more complex than it appears, since an absence of RPE, as occurs in retinitis pigmentosa (498) or sodium iodate toxicity is associated with choriocapillary atrophy (644).

(iii) Aging and Degeneration

Aging changes in the choriocapillaris are remarkable lacking in the majority of eyes examined. Sclerosis of larger vessels is present, but not a prominent feature in normotensive cases (634). The intercapillary distance increases with age, possibly representing some loss of capillaries (623) and the walls become thicker with PAS positive material (370).

Degeneration of the choriocapillaris will be considered at the end of the pathology section, when various pieces of relevant information from other sections of the literature review will be drawn together in a discussion on the pathophysiology of age-related macular degeneration.

Bruch's Membrane

Bruch's membrane in its normal state is acellular, and therefore, the range of pathological changes is limited largely to defects in its continuity, deposition of acellular material, or cellular invasion.

Discontinuity

Bruch's membrane may be ruptured in trauma including photocoagulation, and myopia.

In high myopia, the posterior segment appears to stretch, and the choroid becomes progressively thinned with loss of the choriocapillaris associated with breaks in Bruch's membrane. All breaks in Bruch's membrane are associated with neovascularization, as has been discussed previously. Breaks in Bruch's membrane in myopia are associated with Fuch's spot, which clinically is a small pigmented lesion usually at the macula, and histopathologically is represented by a nodule of hyperplastic retinal pigment epithelium in association with neovascularization of a limited extent (447). It has been emphasized, however, that a break in Bruch's membrane is not a prerequisite for subretinal pigment epithelial neovascularization (503).

A particular form of rupture of Bruch's membrane is described clinically as an angioid streak. A number of histopathologic studies of angioid streaks have been made (96,240,246,409,418,466A,466B,519,569A,617A,892,1012,1156 A). The change is initially a discontinuity of the elastic layer, and later, full thickness breaks, often complicated by choroidal neovascularization. Angioid streaks are associated with a systemic disorder in 50% of

cases (174). Associated diseases include pseudoxanthoma elasticum, Paget's disease, Ehlers-Danlos syndrome and sickle-cell disease (174,438). It has been proposed (174) that a deposition of iron in Bruch's membrane renders it brittle, in some cases, particularly sickle-cell disease, but recently, the brittleness has been attributed to calcification (566) secondary to choroidal insufficiency. Clinically nodular sub RPE deposits are associated with angioid streaks (274A,785A) but have not been described histopathologically.

Ashton described a fundus dystrophy in which he proposed the primary defect to be a defect in Bruch's membrane with secondary chorioretinal atrophy and a disciform response (42).

A classical feature of syphilitic chorioretinitis is rupture of Bruch's membrane, with retinal pigment epithelial cell proliferation filling the defect (92).

Aging and Degeneration

Aging of Bruch's membrane has been widely discussed, as it has long been considered an important factor in the development of age-related macular degeneration.

Bruch's membrane shows ultrastructural changes from young adulthood onward (306). By light microscopy, generalized thickening basophilia and focal deposits have long been recognized (418,507,989). Early ultrastructural studies showed abnormal deposits

(387,460,521,524,695,835,1056,1103) which have subsequently been further characterized by analysis of changes in Bruch's membrane in relation to the morphology of the overlying retinal pigment epithelial cell. It has been found that degeneration of the retinal pigment epithelium, as assessed by accumulation of lipofuscin and melanolipofuscin, directly relates to Bruch's membrane aging changes (306). The conclusion was that the retinal pigment epithelium produces material which accumulates in Bruch's membrane. Hogan has extensively reviewed the early literature and described the ultrastructural changes of age (519). There is a gradual accumulation of extracellular material, frequently showing a vesicular pattern in the inner collagenous zone from age twenty onwards. The origin of the vesicles has been disputed. Hogan (519) believes they originate in the retinal pigment epithelium where cytoplasmic vesicles are frequently seen, while Lerche (695) considered them to be of choriocapillary origin. Other authors also indicated that the vesicles originate in the retinal pigment epithelium (837,978). However, general pathologists recognize degenerative changes in extracellular material with various membrane-like figures seen in basement membranes. These are considered to represent altered type IV collagen, or possibly an abnormal glycoprotein polymerization (415).

From age forty onwards, there is an accumulation of banded collagen in the outer collagenous zone and intercapillary zones, and there may be calcification (519). In older patients, the altered collagens and

membrane-like figures accumulate also on the scleral aspect of the choriocapillaris (Fig. I 13,14). The ratio of collagen fibrils to matrix alters with age with a relative increase in the fibrillar component (309) and a loss of elasticity (322).

In a study of aging monkeys, similar changes in Bruch's membrane have been documented (552) and have been interpreted as budding of effete segments of retinal pigment epithelial cytoplasm. This interpretation is erroneous on the basis of general extracellular material pathology (415).

Basal linear deposit is a material which is found on the inner aspect of Bruch's membrane and in the basal infoldings of the retinal pigment epithelial cells in age-related macular degeneration. Sarks (994) meticulously studied a large group of eyes and classified the aging changes into six groups defined on the basis of the extent of the basal linear deposit. In group 1, there was no deposit, and the other groups progressed from patchy deposits (group 2) through thick continuous deposit (group 4) to group 6 (disciform macular degeneration). She commented that the deposit is a manifestation of failure of the retinal pigment epithelium and is a good indicator of the severity of degenerative change.

Pathogenesis of drusen

Clinically, the term drusen indicates punctate yellow or white deposits in the fundus at the level of Bruch's

Figure I13

Electron micrograph of the choriocapillaris of a patient aged 79. In the inner collagenous zone (ic) of Bruch's membrane are numerous membranous structures, most of which are vesicular. Wide spacing collagen (arrowhead) is present, principally in the outer collagenous zone. RPE - retinal pigment epithelium, e - elastic lamina, CC - choriocapillaris, E - endothelial cell, P - pericyte. (x 16,000).

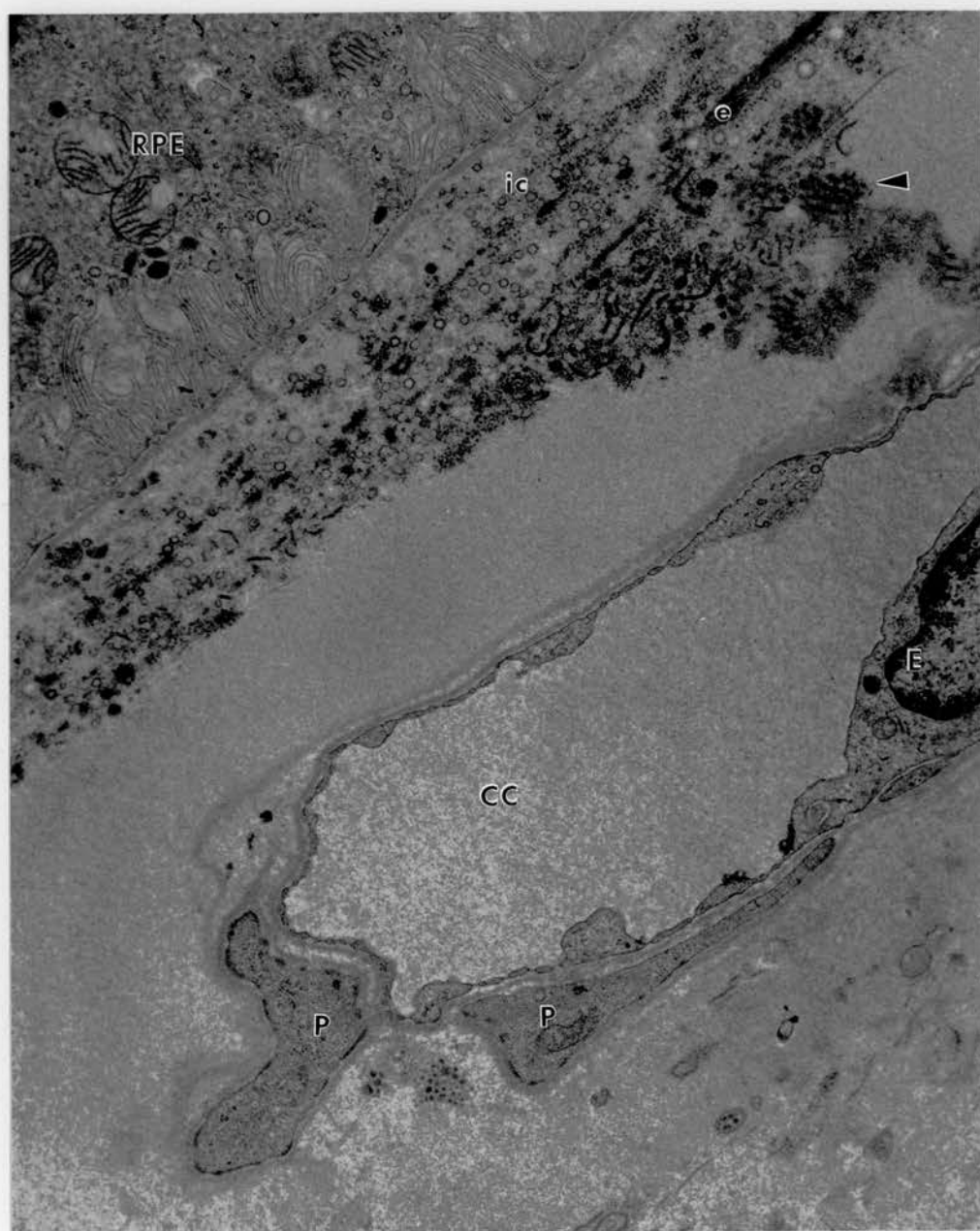


Figure 1 13

Figure I₁₄

Electron micrograph from the same case as figure I₁₄ showing vesicular and curvilinear membrane profiles (arrowhead) on the sceral side of the choriocapillaris (CC). BM - Bruch's membrane, E - endothelial cell, P - pericyte. (x 16,000).

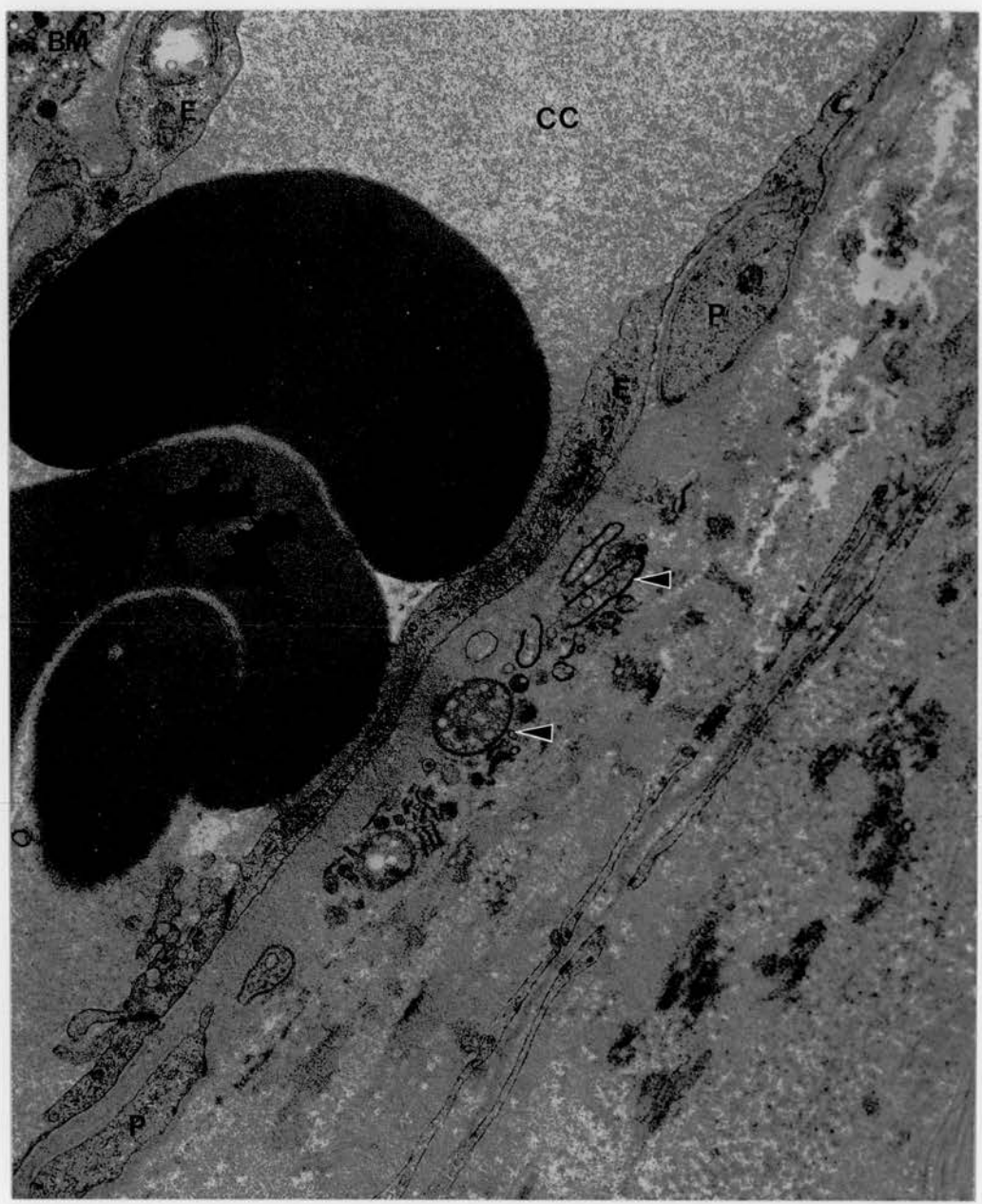


Figure 1 14

membrane. The histopathological definition is imprecise, but is generally considered to be nodular deposits of hyaline material lifting the RPE away from Bruch's membrane.

Drusen are a very common histopathological finding first described in the nineteenth century (241,828). They are present in 87% of the eyes of white patients aged thirty upwards in one autopsy series (181) and 33.5% of eyes of patients aged 0 to 99 years in another series (706). A group of patients included in the second series, aged 26 upwards showed drusen in 56% (705). The discrepancy between the two series may be attributed to a racial difference. The first series did not include blacks who are known to have a lower incidence of age-related macular degeneration (454), while the second did. A racial difference has also been demonstrated in the Japanese who show fewer advanced age related changes than whites (530). In a group of patients over sixty, drusen were present in 100% of eyes (956). However clinically they are seen in less than 25% of eyes of an ageing population (312,615,694). Presumably small lesions detectable on histopathological examination are not clinically apparent. 88% of patients with age-related macular degeneration have clinically detectable drusen (705).

Three types of nodular drusen are commonly recognized (965). These are firstly the type recognized clinically and histopathologically in relation to aging and degeneration, secondly , the type found

histopathologically in scarred or disorganized eyes following trauma, inflammation or neoplasia, and thirdly, the type recognized as being familial.

Drusen of ageing and degeneration change are the type which will be discussed at length. The type found in scarred and disorganised eyes result from reactive change in the RPE which then produces excessive basement membrane material, and may also itself degenerate, contributing to the sub RPE deposit (233,324,879,967,1172).

Familial drusen are histopathologically identical to ageing and degenerative drusen (288), and clinically are considered to be the same by Gass (408) although others do not agree (288,647,846).

There are three longstanding and cyclically favoured theories of the pathogenesis of drusen which have been modified and added to, but remain controversial. Donders (241) believed that retinal pigment epithelial cells were transformed into drusen (transformation theory) while Muller (828) proposed that they were secretory products of intact retinal pigment epithelium (deposition theory). The third theory, the vascular theory, was first proposed by Hofmann (516) who believed that material from the choriocapillaris was deposited in Bruch's membrane. The historical literature has been reviewed in detail by Farkas (287).

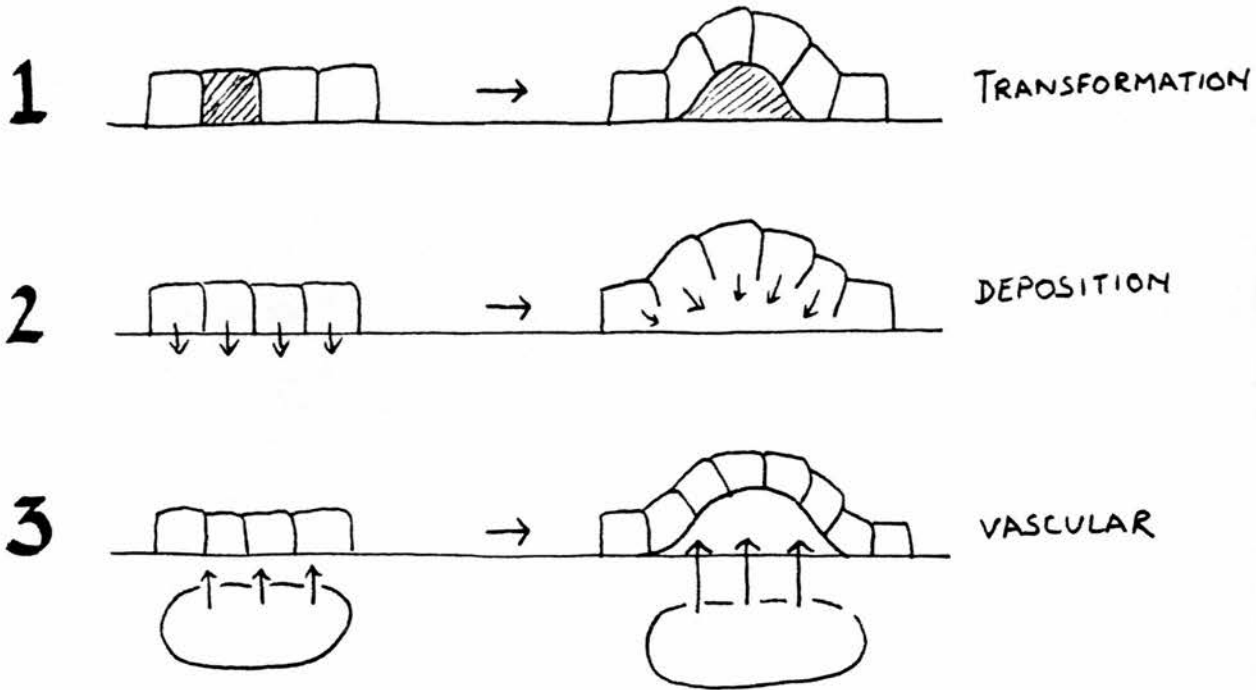
Friedman and coworkers (371,428) substantiated the vascular theory by showing that the distribution of drusen is related to the choriocapillary pattern. Lerche (697) also favored the vascular theory. More recently, Spencer (1056) has suggested that abnormal material accumulates within the retinal pigment epithelium and is then deposited in Bruch's membrane (deposition and transformation theory). Fine and Yanoff (320) believe drusen to be aggregated basement membrane (basement membrane theory) and Burns and Feeney-Burns (137) term their observations of budding retinal pigment epithelial cells the apoptosis theory (Fig. I 15).

The classical description of drusen by light microscopy is of a PAS positive nodular lesion on the inner aspect of Bruch's membrane, elevating the retinal pigment epithelium (519). Different appearances are described at different stages of formation and degeneration and were described in detail by Rones (970) and Wolter (1202). They are shown by histochemical study to be composed of glycosaminoglycans and lipid (291). The glycosaminoglycan is sialic acid, and the lipid a cerebroside (291). Ultrastructurally, drusen consist of a heterogeneous arrangement of vesicular, granular and filamentous material (105,137,290,519). Recently, scanning electron micrographs of drusen have been published (1149). The overlying retinal pigment epithelium may appear abnormal (290) with pigment granule accumulation and mitochondrial degeneration, as well as an accumulation of abnormal basement membrane material between the basal infoldings.

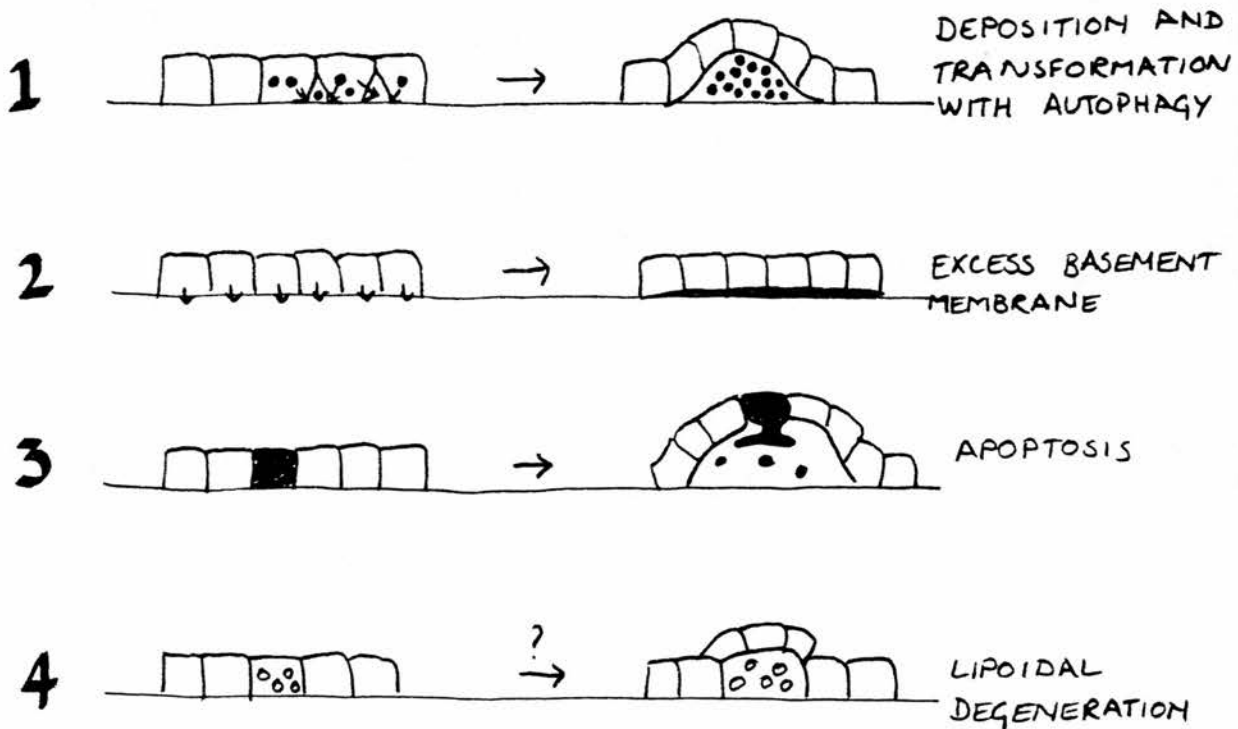
Figure 116

Diagram showing the theories of drusen pathogenesis.

Basic theories



Recent modifications



Pathogenesis of drusen

Figure 1 15

Currently, the most favored theory is the deposition theory, or modified as the apoptosis theory. The evidence in favor of deposition is that the overlying retinal pigment epithelium is degenerate (291,306). Gangliosides, which are composed of cerebroside and sialic acid which are present in drusen, can be stained in the overlying retinal pigment epithelium (291). In familial drusen, there is little doubt that there is a retinal pigment epithelial defect. Retinal function studies, including dark adaptation, ERG, and EOG are abnormal in familial drusen, but not in secondary drusen (287,650), indicating a diffuse abnormality of the retinal pigment epithelium. The morphology of familial drusen and secondary drusen is identical (288), but the retinal pigment epithelium is only focally abnormal, possible accounting for the normal findings on testing of retinal function. (288).

The apoptosis theory of Burns and Feeney-Burns (137) is based on the study of a series of eyes from patients of different ages. They interpreted the sequence of events to be interruption of the basement membrane of the retinal pigment epithelium, deposition of retinal pigment epithelial cytoplasm beneath the retinal pigment epithelium basement membrane, and degeneration of these cytoplasmic packets with drusen formation. This theory has been supported by other workers (551,553) who have shown cytoplasmic processes from retinal pigment epithelial cells, extending into drusen. However, retinal pigment epithelial cells can form cytoplasmic

processes at their bases (518,863,924A,1153) in other pathological circumstances, and the separated cytoplasmic processes are not necessarily from retinal pigment epithelium. Some of the membrane figures designated as cytoplasmic membrane could be interpreted as degenerating collagen (415). In an attempt to create drusen experimentally using intravitreal aminoglycoside injection, retinal pigment epithelial degeneration with formation of subretinal pigment epithelial nodules supports the apoptosis theory (1097).

Aminoglycoside toxicity to the retinal pigment epithelium is characterized by lipid accumulation in the cytoplasm (721). A condition termed lipoidal degeneration or lipidization of the retinal pigment epithelium has been described. Clinically, discrete rounded yellow-white lesions are seen in the fundus (267,318). These are histopathologically represented by lipid accumulation in the retinal pigment epithelial cytoplasm (267,318). A similar lesion has been seen in monkeys (308,319) and has been thought to be pathogenetically related to drusen formation (267).

A basic problem with the apoptosis theory lies in an absence of other morphological changes in the RPE of the process of apoptosis. Apoptosis is a process by which individual cells are removed from tissues, and is in contrast to necrosis, in which sheets of cells are destroyed. Apoptosis is an essential part of tissue development and remodelling and is brought about by recognized alterations in the DNA of individual cells

(605,1216). Ultrastructurally, apoptosis is characterised initially by loss of cell junctions and membrane specialisations such as microvilli. The cytoplasm condenses and the nuclear chromatin marginates. The nuclear membrane becomes indented, with fragmentation of the nucleus. The cell volume is reduced with dense packing of organelles and the cytoplasmic membrane is convoluted. Despite the frequent findings of drusen and the numerous ultrastructural studies of RPE, such appearances have never been described. In addition, the number of RPE cells rises with age (1136) making it unlikely that apoptosis makes a significant contribution to the life span of RPE cells.

Other evidence supporting the deposition theory rather than the apoptosis theory is observed from the process of autophagy, by which effete cytoplasmic organelles are destroyed and excreted (951,1241). The products have been shown, in the frog, to undergo exocytosis into the lateral intercellular space, to migrate to the basal infoldings and to be released into the choriocapillaris (978). Indirect evidence that this process exists can be drawn from studies of Chediak-Higashi cats (195). In these animals, there is an abnormality of membranes with an unusually high fatty acid content. In the retinal pigment epithelium, the lysosomal system fails to break down the phagocytosed outer segment membrane material, and PAS positive bodies accumulate in the retinal pigment epithelium and in drusen-like mounds under the retinal pigment epithelium.

In contrast to drusen, basal linear deposit is intimately related to the retinal pigment epithelial cells which are always markedly degenerate. Basal linear deposit replaces the basement membrane of the retinal pigment epithelium, rather than lying external to it as do drusen (994). This material is also found in relation to retinal pigment epithelial lipidisation (267) and was described in a case of familial drusen (1205) and in choroidal involvement with leukaemia (1205). In the last two cases, it was interpreted as being an exudative response to the choriocapillaris. More recently, a case of "diffuse drusen" has been published in which a deposit similar in distribution to basal linear deposit, but different in structure was seen. The deposit in this case contained complex tubular structures, and was associated with subretinal pigmented epithelial neovascularization (604). It is probably a variant of basal linear deposit.

Retinal Pigment Epithelium

The retinal pigment epithelium shows a range of pathological responses, reflecting the complexity of the morphology and function (253,361,744). By considering the pathology from the histological aspect, many confusing areas of clinical nomenclature can be avoided. The classification to be followed is shown in Table I 2.

Table I 2

PATHOLOGICAL RESPONSES OF THE RETINAL PIGMENT EPITHELIUM

CONGENITAL Disordered growth - aplasia

hyperplasia/hypertrophy

hamartoma

Disordered pigmentation - hypopigmentation

hyperpigmentation

ACQUIRED Disordered growth - atrophy

hyperplasia

"metaplasia"

neoplasia

Disordered pigmentation - hypopigmentation

hyperpigmentation

Disordered cellular function

Metabolic toxic

Inflammatory

Disordered motility and phagocytosis

HEREDODEGENERATIONS AND DYSTROPHIES

AGEING AND DEGENERATION

(i) Congenital

Disordered growth

Aplasia. The retinal pigment epithelium is congenitally absent over uveal colobomata and frequently is hyperplastic at the margins (255,361,388,620,621). It is also found in association with retinal dysplasia (914,1034).

Hyperplasia and hypertrophy. Congenital hypertrophy of the retinal pigment epithelium was classified as such by Buettner (126). Previously, the other terms, including benign melanoma (944) had been used to describe the lesion. The congenital nature of the condition is presumptive in that it is usually static, although progression has been documented (862,1189). Clinically, flat, solitary discshaped heavily pigmented lesions are found in the periphery of the fundus. Characteristically, they have a scalloped edge with a halo of depigmentation. Histopathologically, there is hyperpigmentation and hypertrophy of the retinal pigment epithelium (126,660) as well as hyperplasia with multiple layers of retinal pigment epithelium (1189) underlying an atrophic outer retina. The appearance is contrasted with that of the reactive lesions such as are seen over choroidal tumours (1172) or that of true neoplasia of the retinal pigment epithelium (339).

Hamartoma is defined as a collection of an excessive number of cells normally present in that tissue due to a fault in embryonal development (1175). There is confusion in the literature in the terminology of hamartoma of the retinal pigment epithelium. The lesion described above as congenital hypertrophy of the retinal pigment epithelium has been termed a hamartoma. The term should be limited to a well-defined clinical entity which presents symptomatically in young adulthood when the patient complains of blurred vision. There is a slightly elevated finely granular nodule in the peripapillary region of the fundus (821,973,999). Histologically, the malformation is made up of retinal pigment epithelial cells, glial cells and blood vessels. Contraction of the glial cells causes wrinkling of the retina, and accounts for the blurred vision on presentation.

Disordered pigmentation

Hypopigmentation is encountered in Chediak Higashi syndrome (571,964,1057), albinism (160,381,867) and grouped pigmentation (713,1027).

Hyperpigmentation is the hallmark of grouped pigmentation which is of no clinical significance (362,713,1027).

Francois has classified a large number of teratogenic factors, including infections, deficiency states, toxins and ionising radiation, which may cause congenital retinal pigment epithelial dystrophies, usually characterised by a salt and pepper appearance (350). The commonest of these is rubella retinopathy in which the

retinal pigment epithelial cells are patchily hyperpigmented (101,1195,1226,1253). A lesion termed congenital discoid hypertrophy has also been described, in which a disc-shaped flat lesion is found to be made up of hyperpigmented but not hyperplastic retinal pigment epithelial cells (362,660). This is probably a variant of grouped pigmentation.

(ii) Acquired

Disordered growth

Atrophy of the pigment epithelium is a widely recognised phenomenon occurring in a multitude of conditions including age-related macular degeneration (620), central areolar choroidal sclerosis (39,315,534), retinitis pigmentosa (254,265,390,631,720,793,817,968,991,1094,1095,1156,1201) choroideraemia (775), inflammations (1200), including rubella retinopathy (101,1253) subacute sclerosing panencephalitis (102) cytomegalovirus retinopathy (236) and over choroidal tumours (1172). It is also a feature of a number of peripheral degeneration (1083) including paving stone degeneration (795) and overlying drusen (1200) as well as resulting from toxic (671,1256) and light damage to the eye (468,780,1143,1251,1258). It may be iatrogenic (238,751,1042,1093,1152), resulting from photocoagulation or other excessive electromagnetic radiation exposure (467,550,891,957).

Acute loss of the retinal pigment epithelium can be produced experimentally by occlusion of the posterior ciliary artery (490), by infarction in accelerated hypertension (624) with liquefactive necrosis (1122), or in severe photic injury (1174) when the retinal pigment epithelial cells completely disintegrate within twenty-four hours of the insult, inducing coagulative necrosis (1122). These effects are not identified clinically because of the opacification of the overlying retinal layers which would occur coincidentally in such injuries.

Hyperplasia of the retinal pigment epithelium is a very common response, found in some degree in 59 of 100 consecutive cases submitted for histopathological diagnosis (361). It is found almost invariably in association with patches of retinal pigment epithelial cell atrophy, or in response to any injury at the chorioretinal juncture (361). Typical examples are the pigmented haloes seen surrounding laser (1173,1174) or cryopexy scars, or the tide marks of a longstanding retinal detachment (725,726,727). Fuch's spot seen in myopia represents simple retinal pigment epithelial hyperplasia (447). The proliferative patterns which occur have been extensively described and classified (361,660,945,1121,1128,1142). Clinically the range of lesions is well recognised (660,945). A specific example is the Dalen Fuch's nodule of sympathetic ophthalmia (554). The Dalen Fuch's nodule consists of a collection of epithelioid cells in the retinal pigment epithelium peripherally. These are now considered to be metaplastic RPE cells (448,554) although previously they were

considered to be macrophages from the choriocapillaris. Experimentally, the healing of the retinal pigment epithelium after focal injury such as laser coagulation is by flattening and sliding of adjacent retinal pigment epithelial acells followed by mitotic activity at the margin of the injury (752,1142). The new cells are usually hypopigmented but maintain the blood eye barrier (132,555,1043,1173,1174).

In more severe injury, such as occurs in an intense photocoagulation or cryopexy lesion, the retinal pigment epithelium reacts more markedly and forms a papillary proliferation growing into the retina forming a chorioretinal adhesion (555,1173,1174). If the retinal pigment epithelium loss is widespread, there may be a failure of the retinal pigment epithelium to regenerate, in which case Muller cells and glial cells form a chorioretinal scar (1172). When the lesion is associated with traction, as occurs at the margin of a retinal detachment, the retinal pigment epithelium proliferates more profusely (23) and if encircling the eye, behind the ora serrata, as it does in a total retinal detachment, is termed ringschweile. The retinal pigment epithelium contributes to subretinal bands, a cause of failure of retinal detachment surgery (298,1076).

The more florid proliferative patterns are associated with the production of basement membrane-like materials which stain PAS positive and are described as hyaline or cuticular masses. The simplest pattern produces a drusen-like deposit, covered with retinal pigment

epithelial cells, but more complex laminated and tubular patterns are frequently seen, especially in disciform scars at the macula and in retinal detachments, respectively, and may be misdiagnosed as tumours (314,360,565,944).

A rare form of hyperplasia of the retinal pigment epithelium has been described, in which the retinal pigment epithelium proliferates into the retina in cords in the peripapillary area. This condition is seen in young adults and may be congenital (1107).

Retinal pigment epithelium in long term culture eventually grows in a multilayer pattern, which may be a model for pathological hyperplasia (851). It is suggested that the stimulus for retinal pigment epithelial proliferation is derived from a growth factor produced by the retina (150).

"Metaplasia" is defined as the change of one type of differentiated tissue to another type of similarly differentiated tissue (1175). In general pathology, it is disputed as to whether a cell from one embryonic cell layer can differentiate to a cell from another layer. The forms of metaplasia attributed to the retinal pigment epithelium are fibrous and osseous, both of which imply a change from a tissue of neuroectodermal origin to a tissue of mesodermal origin. Although such paths of differentiation occur in lower animals (1175), there is no evidence that they occur in humans. The first report of "metaplasia" in the retinal pigment epithelium was in

1882 (111) and has subsequently been described particularly in relation to disciform scarring at the macula (523,528,670,729,954), but also in other injuries (523,1120A). However, in contrast to true fibrous tissue, the "metaplastic" tissue contains quantities of PAS positive basement membrane-like material, belying its epithelial origin. By electron microscopy, more epithelial characteristics including desmosomes are identifiable (1120A). It has also been suggested that metaplastic retinal pigment epithelial cells may become epithelioid cells (554).

Autotransplants of retinal pigment epithelium into the vitreous of the fellow eye of monkeys has been used as a method of studying the ability of the cells to transform to cells manifesting nonepithelial characteristics (738,826).

Osseous metaplasia has also been the subject of controversy. Bone formation, sometimes with marrow is found in degenerated eyes (660). Formerly, it was believed that the bone originated from the choroid (627,1051), but Frayer (361) reported that the bone was always internal to Bruch's membrane, if that remained intact. Other authors have suggested that the bone must arise from the pigment epithelium (111,670). This question has recently been reviewed but remains unresolved (283).

Neoplasia. Neoplastic lesions of the retinal pigment epithelium may be benign or malignant. Misdiagnosis is

common both on clinical and histopathological grounds. Histopathologically, the differential diagnosis lies between neoplasia and pseudoepitheliomatous hyperplasia (285,361,384,946,1080,1128). Pseudoepitheliomatous hyperplasia is a reactive response presumably of chronic inflammatory origin, seen in eyes with longstanding disease, and is diagnosed on the basis of the organised arrangement of well-differentiated cells with a regular pattern of basement membrane material. There may be direct invasion of the retina and choroid. Most often, but not always, pseudoepitheliomatous hyperplasia is an incidental finding in chronically diseased enucleated eyes. In contrast, neoplastic lesions show cellular pleomorphism with invasion of the retina or the choroid or both (285,361,660). Neoplasia is rare and occurs in otherwise normal eyes, with a maximum incidence at age 40 years (1121). A variety of growth patterns have been described histopathologically. The cells may be arranged in a tubular or papillary pattern, or be randomly arranged (339,1121). The more anaplastic and pleomorphic tumours are cytologically malignant, but no metastasis or tumour death has been reported (1121), although the malignant potential of neoplastic retinal pigment epithelial cells has been demonstrated experimentally by inducing neoplasia in cultured cells, and implanting them subcutaneously in a guinea pig (12).

Disordered Pigmentation

Hypopigmentation is seen as a consequence of healing of retinal pigment epithelial lesions, as for example,

following laser burns (26,555,1412,1170,1174), or infarcts of the retinal pigment epithelium in malignant hypertension (624). Clinically, the integrity of the retinal pigment epithelial layer can be proven, in the presence of a hypopigmented area in the fundus, by fluorescein angiography. When the pigment epithelial layer is complete, there is no leakage of fluorescein, although a window defect is present (256). It has been observed that focal loss of melanin granules in association with sclerosis of the choriocapillaris may occur as a degenerative change (603).

Hyperpigmentation in its acquired form is due to an increase in the number of lipofuscin granules within the cell, and not melanin granules (338,934,1172). Focal hyperpigmented lesions may be confused with malignant melanoma of the choroid (314,944).

Disordered Cellular Function

Metabolic. An example of a functional defect of the retinal pigment epithelium of metabolic aetiology is the pigment epitheliopathy recognised in diabetes mellitus (252,459,525,612,1131,1132).

There is a large body of evidence indicating that a failure of the retinal pigment epithelium to handle the metabolic load imposed on it by the mechanism of photoreceptor disc shedding may result in pigment epithelial dysfunctions including dystrophies and age-related macular degeneration (520). In cystinosis,

crystals have been identified in the retinal pigment epithelium, seen clinically as shiny yellow deposits at the macula (990).

Primary oxaluria is clinically associated with "flecked retina" (437). Oxalate crystals have also been identified in retinal pigment epithelial cells in a patient suffering toxic effects of methoxyflurane anaesthesia (127,128) and in an experimental model (142). Other anaesthetic agents have been shown to be toxic to the retinal pigment epithelium in laboratory animals (21,61,574,575,1154).

In a variety of storage disease, including lipid storage disease (885), mucopolysaccharidosis (777) and ceroid lipofuscinosis (426) abnormal deposits of metabolites are found intracellularly in the retinal pigment epithelium.

Giant melanolysosomes fill the retinal pigment epithelial cells, in Chediak Hiagashi syndrome, already mentioned in the section of hypopigmentation, suggestive of a metabolic defect (964).

Toxic. Drug toxicity affecting the retinal pigment epithelium is common. The first clinical description of chloroquine retinopathy was published in 1959 (514). Characteristically, there is a bull's eye lesion with central depigmentation and a ring of pigmentation (36,269,499,513). Histopathological studies have been published (67,818,938,939,974,1185) reporting retinal pigment epithelial atrophy and loss of melanin.

Chloroquine binds to melanin, causing a disturbance in the retinal pigment epithelium resulting in loss of function of photoreceptor cells (1,68,711,1259). It is suggested that chloroquine first binds to sites in the choroid (423) and then, by stabilising lysosomes (1182), impairs phagocytosis (1). Chloroquine retinopathy is now rarely seen because the doses used clinically are much reduced compared with former times (326).

Other drugs are known to preferentially bind to the pigment epithelium. One well recognised group is the phenothiazines (611,711,1179). Clinically, there is coarse mottling of the macular area. Histopathological study shows a primary effect on the retinal pigment epithelial photoreceptor complex, with secondary choroidal atrophy (802). In an experimental model, lipid has been found accumulated in the retinal pigment epithelium (785).

Antibiotic toxicity has been demonstrated in animals (779). Gentamicin has been shown to produce an alteration in lysosomal function in the retinal pigment epithelium of rabbits, with retinal epithelial necrosis and hyperplasia (216) and aminoglycoside toxicity has recently been developed as a model for drusen formation (1097).

Metals are known to be toxic to the retinal pigment epithelium. Iron may be introduced in systemic siderosis or as a foreign body in trauma cases. Clinically, iron causes a fine pigmentation at the macula

(171,172,441,594,1168) which has been studied histopathologically in experimental models (56,221,758,1234). Systemic lead poisoning is recognised as causing a retinal pigment epitheliopathy (441), which can be modelled in experimental animals (119,538,992).

Toxic chemicals have been employed experimentally to induce lesions in the retinal pigment epithelium as models of human disease (859), particularly sodium iodate (457,855,1054,1235).

Inflammatory. Infections of the retinal pigment epithelium include rubella, measles, and syphilis. In congenital rubella, there is scattered pigmentation, described as salt and pepper fundus (350), which may increase in time (788). Histological reports indicate that the pigment epithelium is patchily nonpigmented, but forms a uniform monolayer (101,1195,1226,1253).

Measles may cause a congenital or an acquired retinopathy. In utero infection may result in a pigmentary retinopathy similar to rubella retinopathy (464,791). Measles acquired in childhood may also cause a retinopathy (349,500,948,1005) and subacute sclerosing panencephalitis gives a different pattern (444,870,960) with inclusion bodies in retinal neurones and a secondary retinal pigment epithelial involvement (844).

Disordered Motility and Phagocytosis

As was noted in the section on physiology, retinal pigment epithelial cells have a very important function in phagocytosing other segment discs. They also function as phagocytes in a pathological response to injury. This function has long been recognised (132,180,220,361,671) and has been the subject of experimental investigation (13,26,108,263,270,382,476,527,918,949,950). A defect in phagocytosis has been suggested as the primary lesion in retinitis pigmentosa, from study of the RCS rat, but is not substantiated in the human (108). Phagocytes in the retina may also arise from the retinal and choroidal circulations (13,424). When the retinal pigment epithelial cells are phagocytic in response to injury, they migrate away from their position on Bruch's membrane into the subretinal space (301), retina, or vitreous, if there is a breach in the inner limiting membrane of the retina. The cells may migrate individually or in sheets as they may do through retinal tears, contributing to preretinal membrane formation (361,455,512,660,677,726,1223).

In retinitis pigmentosa, the clinical hallmark is due to retinal pigment epithelial cell migration into the retina, although the relationship of this feature to the aetiology and pathophysiology of the functional defect is obscure (54,429). RCS rats have been used as a model for retinitis pigmentosa, and are known to demonstrate a failure of phagocytosis (98,144,506,827), although the model does not fit the disease fully.

iii Hereditary degenerations and Dystrophies

A large number of disorders affecting the retinal pigment epithelium either primarily or secondarily cannot be classified because insufficient information is available. A number of these are hereditary, but others which are not are presumed to be caused by an unidentified insult. One of these conditions which is of some interest has been termed lipoidal degeneration. This was first described in monkeys (308,318,319). Lipid has been identified filling individual retinal pigment epithelial cells. Subsequently, similar abnormalities have been described in man (267,603) with histopathological studies confirming the similarity to the lesion in monkeys (267). This lesion is of interest because it may be a precursor of the lipid containing form of nodular drusen previously discussed. It may be further studied by experimental induction of the lesion using drugs (216,429) or laser coagulation (1133).

The histopathological features of hereditary macular dystrophies have recently been reviewed (856). In the hereditary group is retinitis pigmentosa which has already been mentioned. Retinitis pigmentosa comprises a group of hereditary diseases characterised clinically by night blindness, variable bone spindle pigmentation of the retina, retinal vascular attenuation and disc pallor, and all manifesting histopathologically at the end stage, retinal pigment epithelium and photoreceptor atrophy with pigment migration into the retina, vascular involution and preretinal glial membranes. The

relationship of classical retinitis pigmentosa to other forms is unknown, but the likelihood is that a large number of individual defects in the retinal pigment epithelial photoreceptor complex could occur, giving rise to a similar end result of a pigmentary retinopathy (254). For example in the RCS rat failure of phagocytosis has already been mentioned as the primary lesion leading to retinal degeneration. In another retinal degenerative condition, lipofuscin accumulation is slower than normal (597) possibly indicating a failure in the disc shedding and phagocytosis process. Related conditions include fundus flavimaculatus (258,348,457,625,846), fundus albipunctatus (457,745), cone rod dystrophy (936), Best's disease (70,354,651,778), Oguchi's disease (781,1221,1222), and systemic diseases such as abetalipoproteinaemia (617) and Kearns's Sayre syndrome (257,781). One clinical type of retinitis pigmentosa is seen in association with Refsum's syndrome, an inborn error of metabolism, in which lipidosis of the tissues is a feature. Histopathologically, a layer of fat is found between the retinal pigment epithelium and the degenerated photoreceptors, which has been interpreted as being a barrier to the supply of metabolites to the outer segments, resulting in their degeneration (1117). Lipofuscin has been shown to accumulate in the retinal pigment epithelium in fundus flavimaculatus (745), cone-rod dystrophy (936) Sjogren Larsson syndrome (854), Best's disease (355,1181) in one case of retinitis pigmentosa (631) and possibly in a type of pattern dystrophy (893).

There are no known dystrophies primarily affecting the retinal pigment epithelium, but a group of conditions termed pattern dystrophies are presumptively put in that category. They are a group of hereditary disorders which progress slowly and reproduce different patterns of pigmentary disturbance symmetrically in the fundi. No histopathology is available (226,231,316,356,536,748,857,864,1038).

In the nonhereditary group, the suggested aetiologies are inflammatory, vascular, photic injury and toxic (747). The commonest of these is acute posterior multifocal placoid pigment epitheliopathy (48,397,1099), but other include serpiginous choroidopathy (49,404,469,883,1003) and acute retinal pigment epithelitis (176,225,266,649,855).

iv. Aging and Degeneration

In degenerative macular diseases, the photoreceptor lesion is often secondary to disease of the retinal pigment epithelium (257,258,758,1184B).

The retinal pigment epithelial cells become progressively more pleomorphic, both in regard to the cells themselves, and the pigment granules, with age. At the macula, the cells become higher and narrower (373,606).

Lipofuscin accumulation is considered to be the end result of a lifetime's phagocytic activity. It

accumulates in all retinal pigment epithelial cells with time (300,305,520,637,701,811,1188). Concomitantly, the melanin content of retinal pigment epithelial cells falls (300,307,1011), and may make a contribution to the pathogenesis of age-related macular degeneration, in that melanin is one of the major protections which the retina has against the damaging effect of light. In lower animals this process is influenced by pituitary hormones particularly prolactin (1178). The number of melanolysosomes and complex granules also increases with age to a most marked extent at the macula (307). The end result being a greater proportion of the cytoplasm being occupied with pigment granules in retinal pigment epithelial cells from old, then from young subjects. The accumulation of membrane-bound inclusions in the retinal pigment epithelium does not in itself limit the effective functioning of the cells. In an animal model of a mucopolysaccharidosis, despite very numerous retinal pigment epithelial cell inclusions, the photoreceptors remained normal (5). An experimental model to investigate the role of phagocytosed debris in aging changes at the macula has been developed (107).

The basal infoldings of the retinal pigment epithelium alter with age (520,599) and the cells produce excessive quantities of basement membrane and other collagens. Their contribution to Bruch's membrane deposits and drusen formation is discussed separately.

The aging process can conveniently be studied in mice (810) and rats (598). The principle observations

parallel those found in studies of human tissue in that the lipofuscin content is increased, the apical and basal infoldings are altered and banded collagen and other unidentified extracellular materials are deposited between the infoldings. The lysosomes are unusually large, probably because of a failure of acid phosphatase to digest the content (702). In addition, it has been observed that the rate of phagocytosis of rod outer segments slows with age (598).

4. Age-related macular degeneration

The defects in our knowledge of the pathophysiology of age-related macular degeneration do not allow a complete picture to be drawn from the preceding information on the pathology of the choriocapillaris, Bruch's membrane and pigment epithelium.

The description of the natural history of a lesion at the macula progressing to disciform macular degeneration presented by Gass has stood the test of time (391,393,395,396,400, Fig. I 16). He hypothesized that degenerative changes in Bruch's membrane including drusen formation, lead to subretinal pigmented epithelial neovascularization with leakage of fluid and retinal pigment epithelial detachment. Subsequently there is hemorrhage which breaks through into the neuroretina, organizes and forms a disciform macular scar. The unanswered questions are: what is the nature of the degenerative change in Bruch's membrane; what is the contribution of the retinal pigment epithelium and the choriocapillaris; and what is the stimulus for neovascularization?

A number of classifications of "drusen" exist to include the variety of clinical and histopathological findings encountered. Sarks (995) defined four groups - hard, soft, semisolid and regressing. Green (451) termed his four types hard, soft, calcified and diffuse, and Tso (1127) called them hard, soft, papillary and diffuse. Gars only recognises two types - cuticular or basal

Figure 116

Diagrammatic representation of the evolution of the disciform scar.

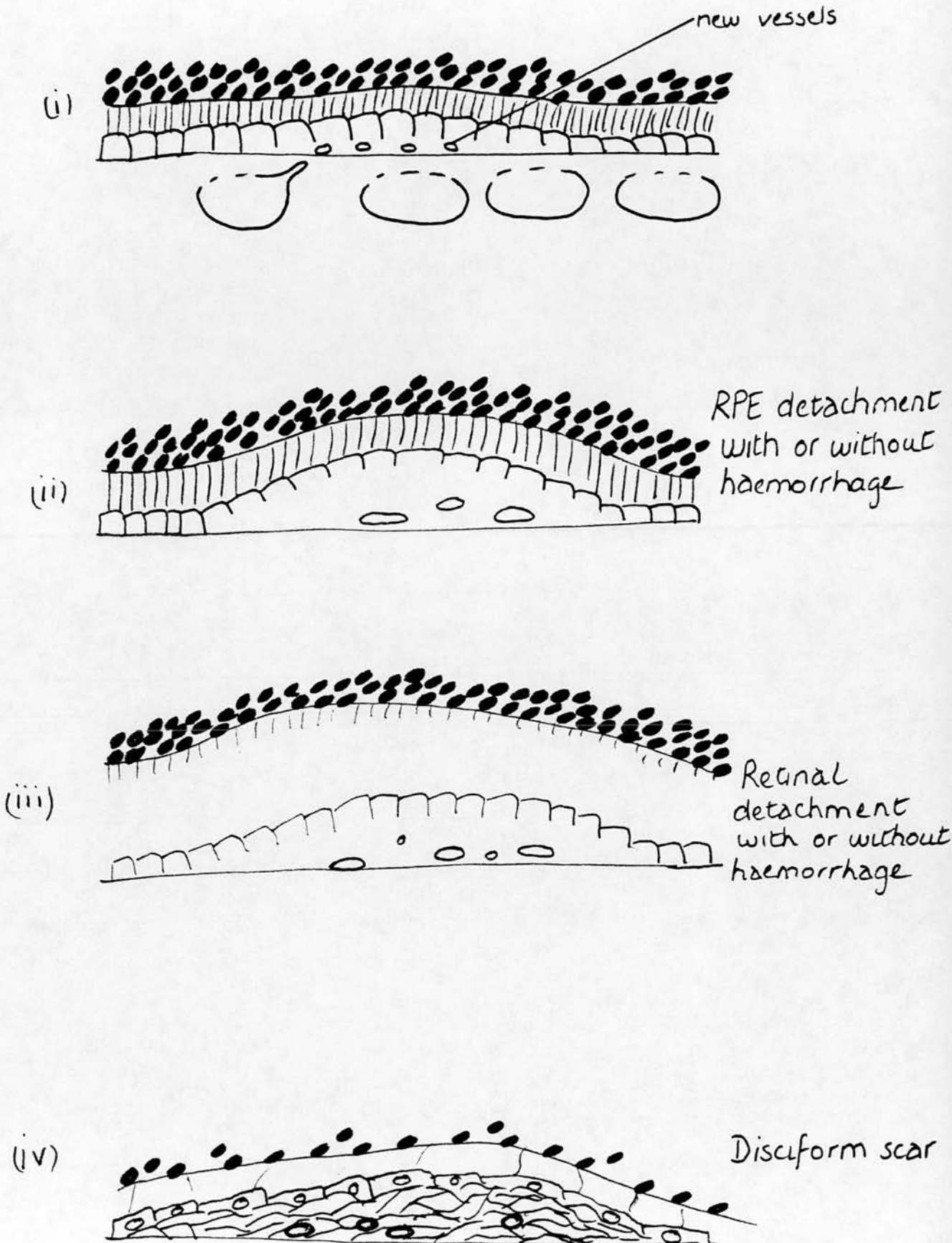


Figure 1 16

laminar and nonculticular or typical. The classification will be discussed further in Chapter II, III and IV.

While "drusen" themselves do not give rise to visual loss (399), they are closely associated with age-related macular degeneration, an association recognized for fifty years (1157). However, in the clinical literature on the subject of drusen, the type has not been related to the visual prognosis. Histopathological studies indicate that nodular deposits under the retinal pigment epithelial basement membrane, on the inner aspect of Bruch's membrane, are not associated directly with age-related macular degeneration. So-called "soft" drusen are the type which increase with age and are related to age-related macular degeneration (181,705). "Soft" drusen are deposits which replaces the basement membrane of the retinal pigment epithelium, and probably represents abnormal basement membrane production (451,994,995,997). They do not fit the general definition of "drusen" in that they are not under the basement membrane of the RPE. This deposit represents a general disorder of the retinal pigment epithelium, which may be the primary disorder in age-related macular degeneration. When the deposit, be it termed basal linear deposit, soft drusen, or confluent drusen, is extensive, it becomes more fluid, possibly by disturbing the osmotic pressure difference required for fluid to be resorbed into the choriocapillaris and the lesion is then clinically recognized as a retinal pigment epithelial detachment (451,767,997,1040). In this state, the

retinal pigment epithelium is atrophic accompanied by gradual loss of photoreceptors.

Hogan (519) believed that thickening of Bruch's membrane was the primary change in age-related macular degeneration. Some authors considered the thickening due to chroidal exudation (385,391). Gass (391) thought that the submacular capillaries were exposed to a higher perfusion pressure, explaining the prediliction for degenerative change to occur at the macula. Alternatively, venous obstruction has been proposed as a cause of choriocapillary exudation (374,385).

A hereditary factor may also operate (622,636) and the degeneration has consequently been termed an abiotrophy. Francois (351) reviewed the literature in the familial features of macular degeneration. Four reports of identical twins which have developed disciform macular degeneration have been published (400,787,1166,1167).

The factor which dictates whether or not neovascularization occurs is unknown. It has been observed that surviving retinal pigment epithelium always accompanies new vessels (806) although the retinal pigment epithelium may survive because of the presence of vessels rather than be a necessary factor in their development. An inflammatory mediator has been suggested, and mononuclear cells are found in the stroma of the choroid of disciform lesions (261,609,899,900,901) and elsewhere in the body, such cells stimulate vascular proliferation (926). However, macrophages are only

present in the choroid and sub RPE space in advanced disciform lesions (106).

Possible factors inducing neovascularization are ischemia due to closure of chroidal capillaries and intercapillary sclerosis (618,1157) or inner retinal ischemia (261,639). Neither can be the answer, since, as previously stated, the choriocapillaris remains remarkably healthy in age-related macular degeneration (636), and experimental occlusion of the retinal circulation does not induce subretinal neovascularization (33,805). Other possible stimuli are light damage (256A) with or without ascorbate deficiency (1127). A generalised degenerative change in elastic tissue is also proposed (103). Numerous histopathological accounts of cases of age-related macular degeneration have been published (137,306,358A,450,451,460,519,530,604,620,622,636,636A,714A,994,995,1040,1057A). In addition to the commonly described features, individual case studies report unusual findings. Occassionally, the subretinal pigmented epithelial neovascular membrane is supplied from the retinal rather than the choroidal circulation or from both (428,429).

The distribution of chorioretinal changes preferentially at the temporal side of the posterior pole has been mentioned previously. One recent concept to explain the distribution of drusen at the macula suggests that there is an anteroposterior drift of accumulated debris in Bruch's membrane, as well as an interior to exterior movement. The anteroposterior drift is dictated by the

presence of the larger capillary bed in the choriocapillaris at the posterior pole acting as a sink (474), (Fig 117). Another theory which receives very little attention is that the pattern of cisterns in the vitreous somehow concentrates whatever toxic metabolites may be present, in the bursa premacularis, the part of the vitreous immediately overlying the macula (1208,1209).

In one epidemiological study, hypertension was related to the extent of drusen formation (1160) but it is not conclusively proven to be correlated with age related macular degeneration (95,544,584). One study has shown an excessive drop in blood pressure in the ophthalmic artery in patients with age related macular degeneration (734). In contrast, one theory of pathogenesis of age related macular degeneration is an abnormally high pressure in the subchoroidal arteries (81,930).

Moving a step backwards from discussing the morphological changes, researchers have been looking for the agents which cause the biological events resulting in the structural changes observed histopathologically and clinically. Much attention has been directed to the damaging effects of electromagnetic radiation. Visible light is known to produce macular lesions (62,369,458,515,550,662,665,674,689,753,754,756,780,861,868,877,891,971,1134,1137,1141,1143,1243). This hazard is recognized as a problem associated with the use of ophthalmic instruments (145,380,712,776,961) and has been known as a cause of acute damage in sungazing (657,1137).

Figure I,7

Diagram showing the possible drift of debris from the retina to Bruch's membrane centred on the macula.

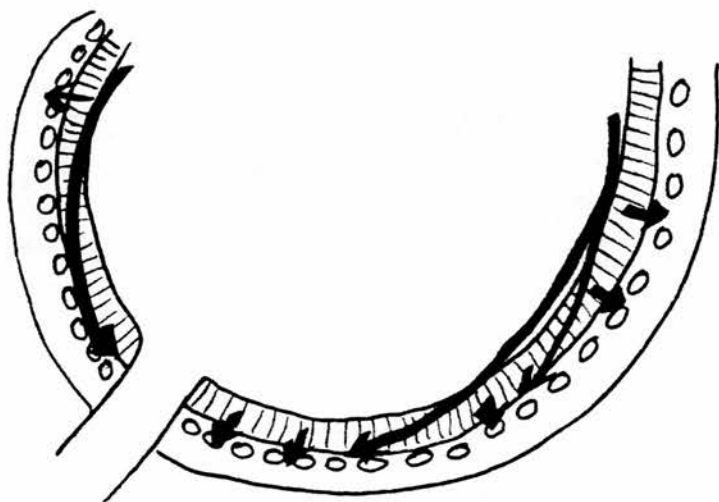


Figure 117

From study of such naturally occurring injuries and experimental studies, it has been extrapolated that in the course of a lifetime, the exposure to ambient levels of light may have a cumulative deleterious effect on the macula. Light interacts with oxygen in the retinal pigment epithelium, generating free radicals which cause peroxidation of lipids in membranes. Vitamin C is a collector of free radicals and is known to protect the retina of experimental animals from the damaging effect of light (709). Similarly, Vitamin E deficiency is associated with the development of macular degeneration (478). This may be the mechanism which leads to the accumulation of lipofuscin in aging retinal pigment epithelial cells. However, in an epidemiological study of age related macular degeneration vitamin A and C levels did not correlate with the disease (95). Other possible mechanisms for light toxicity are that retinol produced by photolysis of rhodopsin may itself damage membranes or the physiological response of the photoreceptor may be transformed to a degrading effect in excessive light exposure (860).

Young (1243) has drawn up a theory of macular disease based on a review of a large body of experimental work. He draws a number of conclusions, but those of clinical significance are that melanin is protective against photochemical damage, that the threshold for retinal degeneration produced by visible light is lowered with age, that nutritional deficiency of vitamin A is associated with a reduction of the threshold to light damage, that diffuse light damage affects the central

retina more severely than the peripheral retina, and that the lens protects the retina from light damage. However, a degree of protection is also offered to the fovea by the luteal pigment (1049,1050). In a clinical study of the peripheral retinal function, in relation to age, the peripheral retinal functional abnormalities do not correlate with age-related macular degeneration (1092), as would be anticipated from Young's theory.

Electromagnetic radiation outside the visible spectrum also causes degeneration of the retina (732,1251,1258). It is possible that light damage accounts for the exacerbation of macular degeneration reported following cataract extraction (88).

Zinc and copper metabolism has been shown to be disturbed in age-related macular degeneration (1036). The significance of the abnormal zinc levels is unknown although the choroid has the highest zinc concentration in the body (198). Copper participates in melanin metabolism in the retinal pigment epithelium (1037).

Chapter II

Clinicopathological case studies

In this section three clinical correlative studies are presented, each giving an opportunity to study a different aspect of the involvement of Bruch's membrane in disease of the RPE and choriocapillaris. The first is a case of age related macular disease, the second a case of retinitis pigmentosa and the third a case of glomerulonephritis with choroidal involvement.

1. Age related macular degeneration

Histopathological accounts of the age related changes in RPE and Bruch's membrane are numerous and are reviewed in Chapter I. There are also many clinical accounts of the degenerative changes seen at the macula (79A,134A,403,404,412A,747A,846,994,1051A) which will be discussed further in Chapter IV. I have had the opportunity to study the eyes of a patient with extensive aging changes and have attempted to evaluate these in relation to clinical findings.

Report of a Case

An 80 year-old man had a cataract in the right eye, reducing his visual acuity to counting fingers, while the left eye had a visual acuity of 6/9 with a clear lens, and macular drusen with mottled depigmentation of the RPE. He had an uncomplicated right extracapsular cataract extraction with implantation of a posterior

chamber lens. Postoperatively, vision of the right eye improved to 6/18 when "macular drusen and atrophic macular degeneration" were noted in the right fundus. Four months following surgery, there was a right anterior uveitis with macular edema. A fluorescein angiogram at the time Fig II 1,2) showed that both eyes showed focal punctate hyperfluorescence, areas of less intense hyperfluorescence with a shaggy outline, and mottled window defects in the RPE. In the right eye hypofluorescent patches and late diffuse leakage of fluorescein were also seen, interpreted clinically as macular edema in the right eye was persistent over the following months. Eighteen months following surgery, the patient died of pneumonia with cardiopulmonary arrest.

Preparation of tissues and histopathologic findings

The eyes were enucleated one hour after death, opened along the equatorial region, fixed by immersion in paraformaldehyde glutaraldehyde fixative, embedded in paraffin in portions, and were stained with hematoxylin and eosin, and periodic aci-Schiff stains.

Blocks from the foveal area of both eyes were postfixed in Dalton's chrome osmium fixative, dehydrated in graded alcohols, and embedded in epoxy resin (Durcupan). Thick sections stained with Mallory's blue were examined by light microscopy, and thin sections stained with uranyl acetate and lead citrate were examined by transmission electron microscopy (Hitachi 300).

Fig. II 1A. Fundus photograph of the right eye, postoperatively, showing retinal oedema and irregular pigmentation. There is mild vitreous haze.

B. Fluorescein angiogram of the right eye postoperatively showing late leakage of fluorescein.

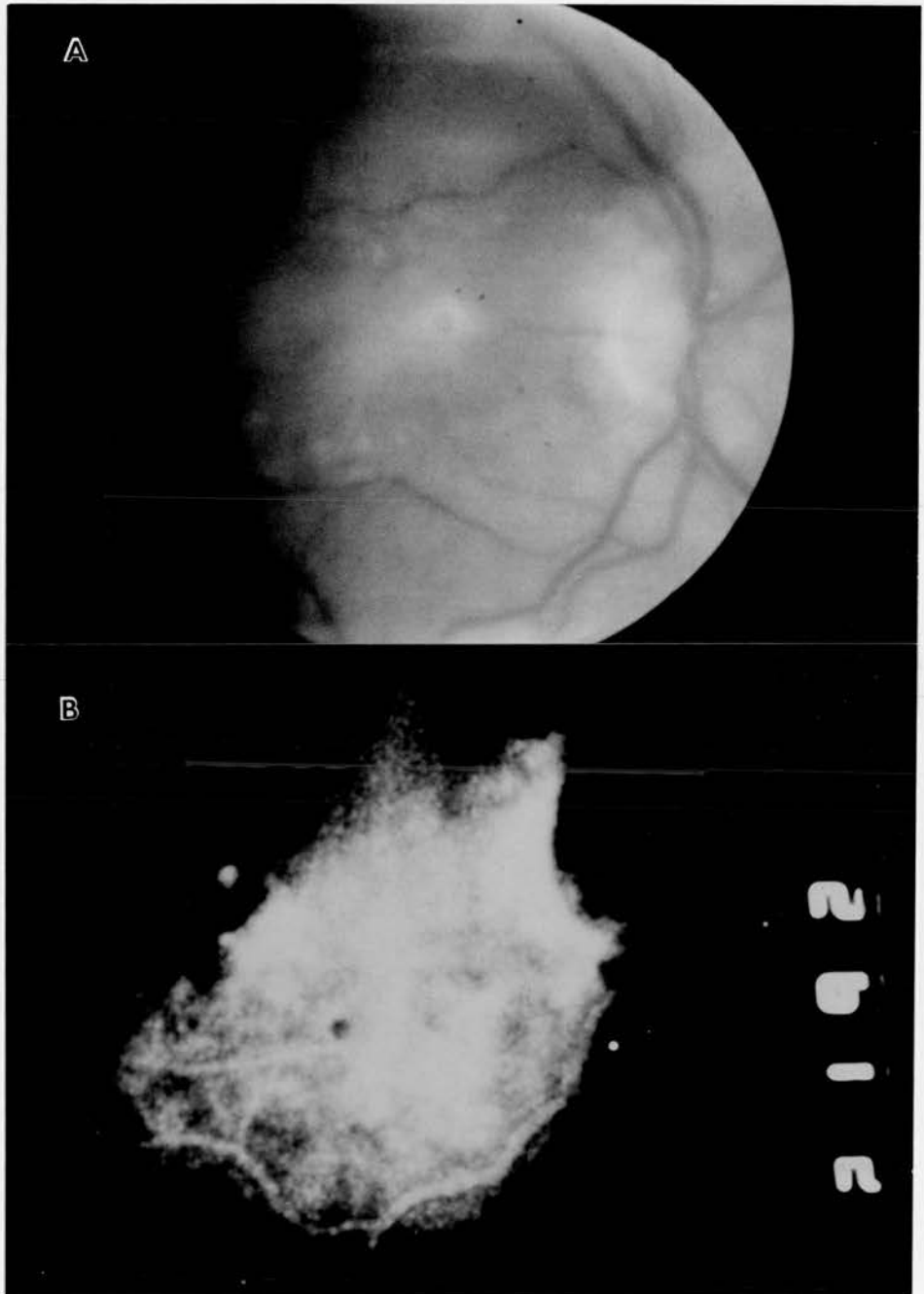


Figure II 1

Fig. II 2A. Fundus photograph of the left eye showing mottled depigmentation of the RPE and punctate deposits. B. Fluorescein angiogram of the left eye showing widespread punctate hyperfluorescence. There is an area of hypofluorescence in the macular area.

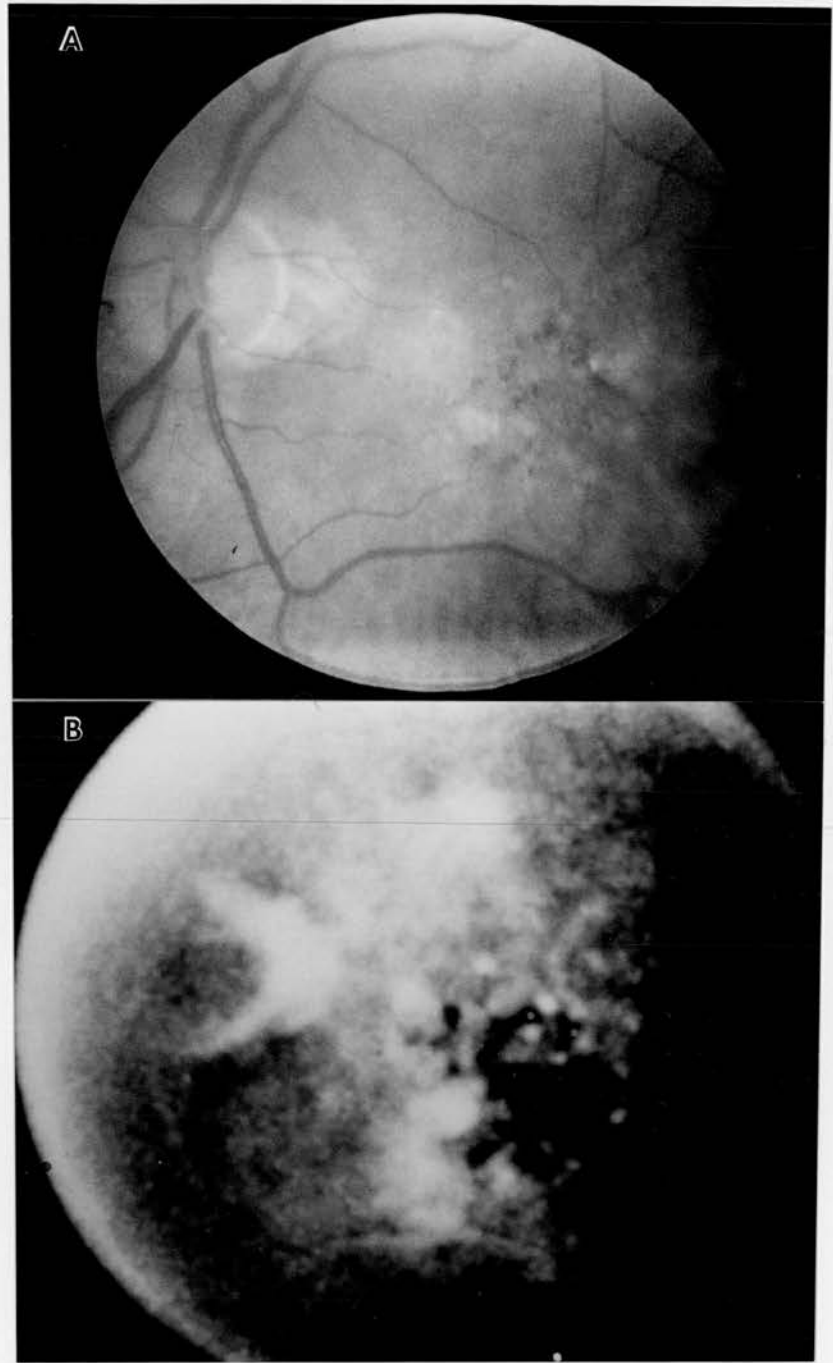


Figure II 2

Results

Light Microscopy

In the right eye there was a healed peripheral corneal scar secondary to cataract extraction. There was mild hyalinization of the ciliary muscle and sloughing of the ciliary epithelium in the region of the lens haptic, associated with mild chronic inflammation. The posterior lens capsule was intact with some fibrous metaplasia of the lens epithelium in the equatorial region. There were some spindle iris stromal cells, pigment epithelial cells, and multinucleated foreign body giant cells on the optic of the lens. The anterior segment of the left eye showed no abnormality.

The abnormalities in the choroid and the retina of both eyes were confined to the macular area, with the exception of some small globular hyaline deposits of typical drusen in the peripheral retina of Bruch's membrane and in the peripapillary region (Fig II 3,4). Typical drusen are deposits of heterogenous material in the inner collagenous zone of Bruch's membrane, separated from the RPE by linear basement membrane. At the macula of the right eye, there was a preretinal glial membrane (Fig II 5). The inner retinal layers were otherwise unremarkable.

In both eyes there was marked loss of nuclei in the outer nuclear layer with some pyknosis. The photoreceptor

Fig. II 3A,B. Light micrographs showing "typical" drusen deposits in peripheral Bruch's membrane. Stain Mallory's blue x 600.

Fig. II 4. Electron microscopically, the RPE (RPE) is lifted up by a smoothly domed deposit (D) of finely granular consistency. The RPE has produced excessive basement membrane (bm) and a linear condensation (arrowhead) outlines the deposit. (x 7,000).

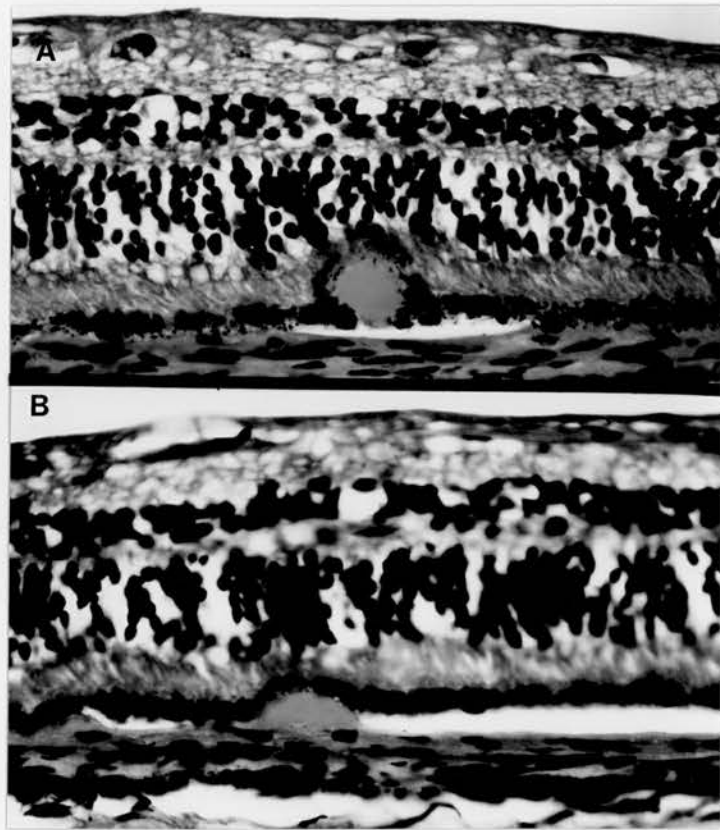


Figure II 3

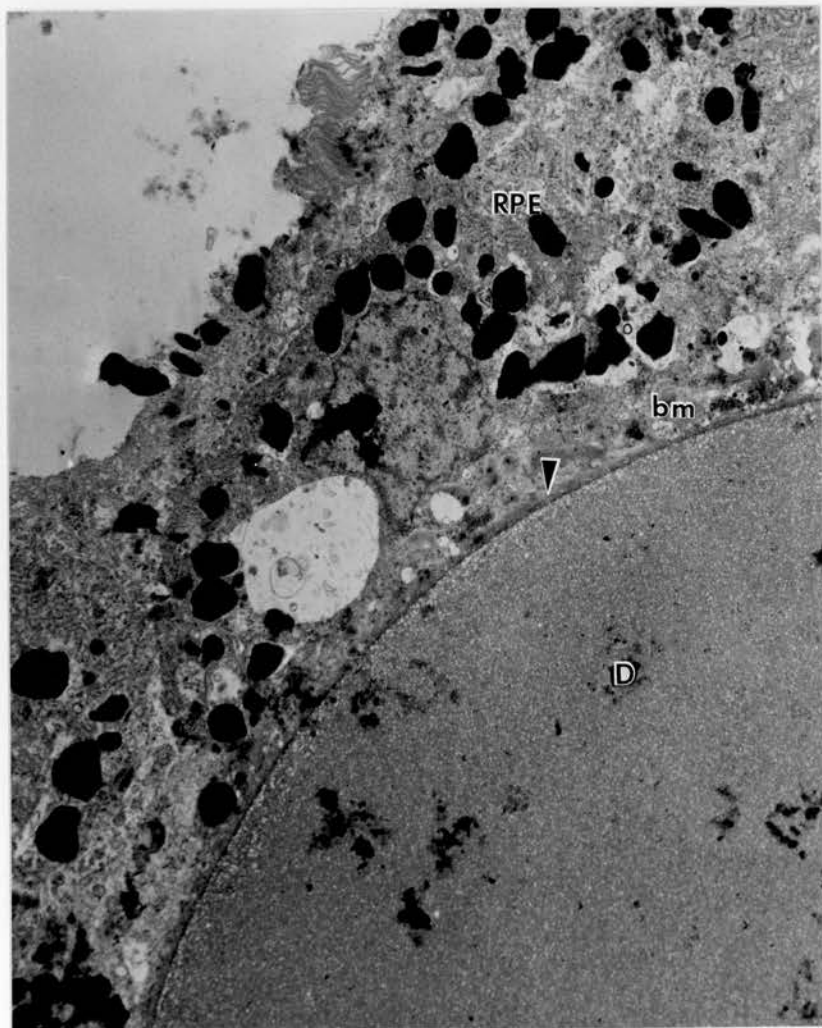


Figure II 4




Fig. II₅. Light micrograph showing preretinal membrane puckering the inner lining membrane. The inner retina is otherwise intact. (PAS x 400).

Fig. II₆. Light micrograph showing double layer of RPE cells (rpe) with basement membrane-like material (d) separating the two layers.

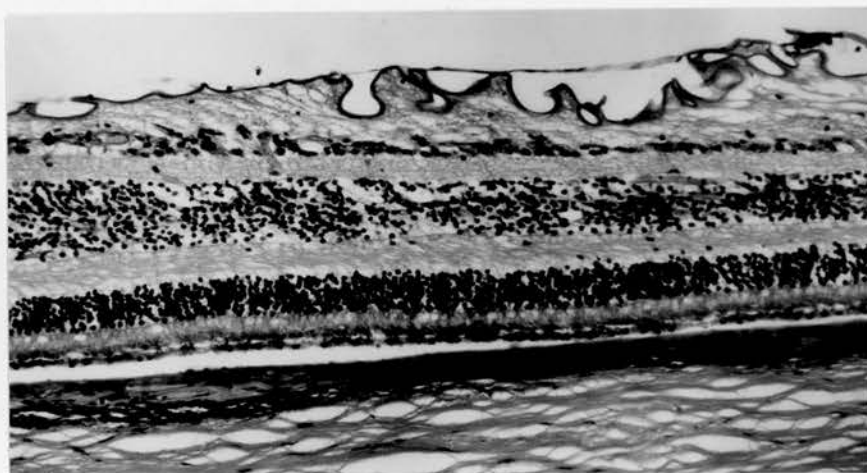


Figure II 5

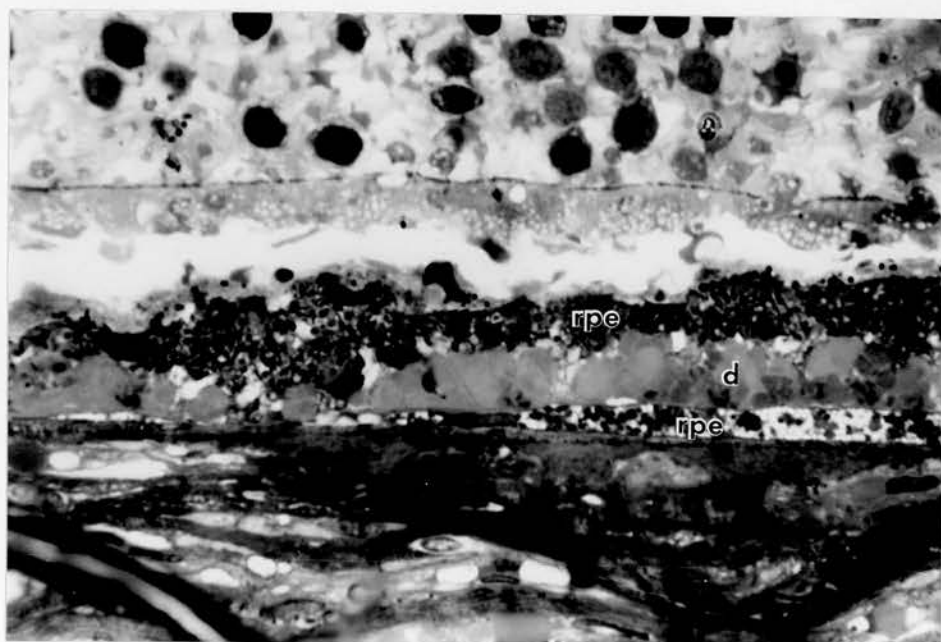


Figure II 6

layer varied in thickness and in some places was completely absent.

The changes in the RPE-Bruch's membrane choriocapillary complex in the the two eyes are to be contrasted, both showing retinal pigment epithelial detachment, but with apparently different pathogenesis.

In the left eye (Fig. II 6) light microscopy showed advanced aging changes in the RPE with irregularity, proliferation and duplication. An extensive heterogenous craggy deposit with clefts and vacuoles was present under the RPE where it had proliferated.

By electron microscopy (Fig II 7,8) the degeneration of the RPE was characterised by loss of the apical processes, with photoreceptor disorganisation, accumulation of pigment granules including melanin, lipofuscin and melanolipofuscin in the cytoplasm, and some vacuolation. In addition, rounded packets of membranous material having a trilaminar arrangement, with round profiles on cross section were identified (Fig II 7A,27). The basal infoldings were greatly widened and filled with banded and vesicular basement membrane. No linear basement membrane was identifiable.

In some areas the RPE was detached (Fig II 9,10). Light microscopically the sub RPE space contained fragments of craggy material similar to that shown in Fig II 6, which remained attached to the RPE in places. By electron microscopy there was a homogeneous moderately dense

Fig. II7A. Electron micrograph showing multilaminar arrangement of the RPE (rpe) with excessive basement membrane-like material deposition (d) between cells, note the multilaminar membranous material (arrowhead). However, no laminar basement membrane is seen. Distorted photoreceptor outer segments (OS) are noted. BM - Bruch's membrane. (x 7,000).

Fig. II7B. In another area, a more extensive deposit (d) sometimes with wide spacing collagen (arrowhead) is present under the markedly degenerate RPE. No photoreceptor outer segments are present. (x 7,000).

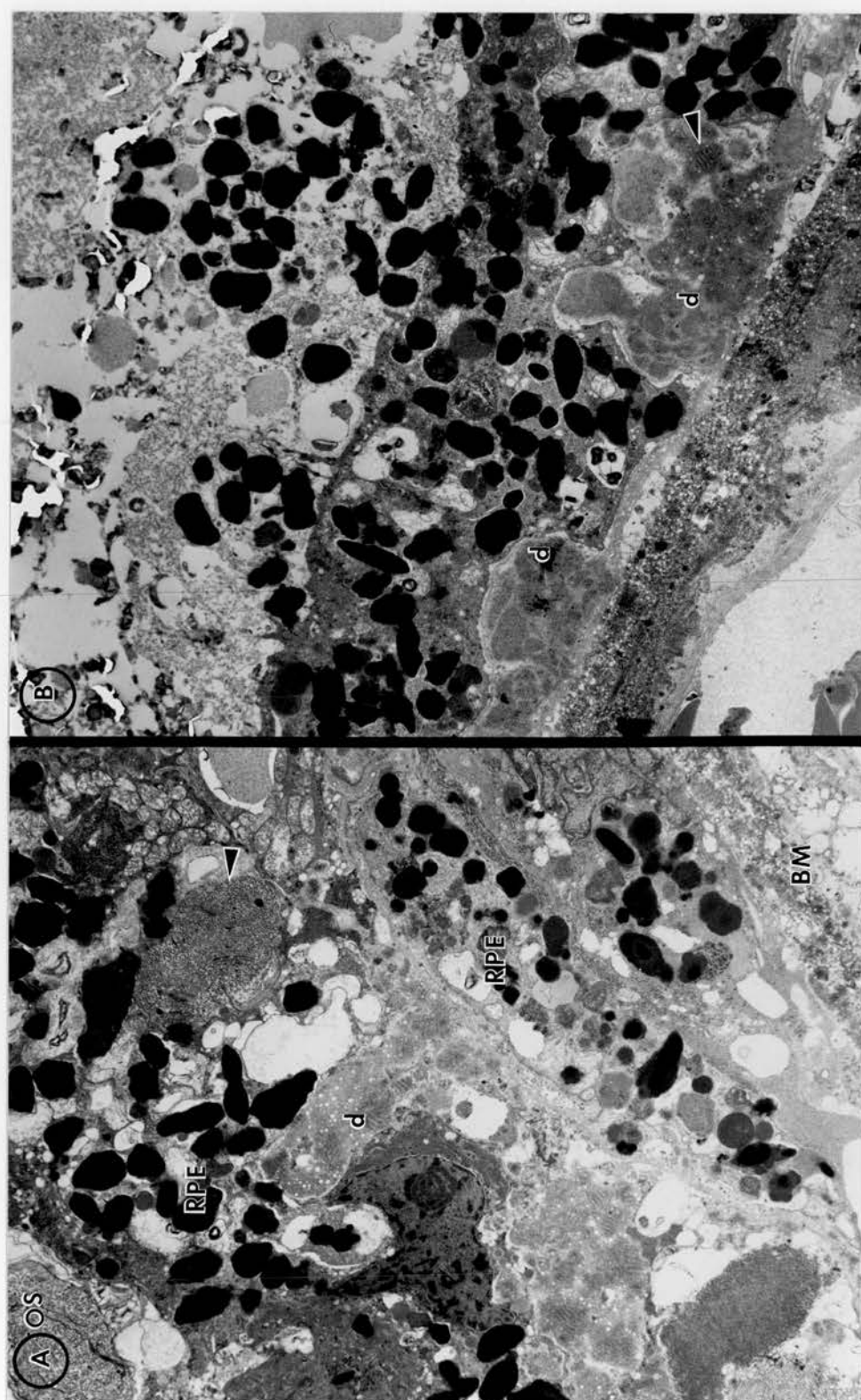


Figure II 7

Fig. IIa. Electron micrograph showing craggy deposit associated with marked RPE degeneration with lipoidal granules (l) and distorted membranous granules (m), possibly phagosomes. The RPE (RPE) forms two layers. Only a few distorted photoreceptor outer segments (OS) are present. x 7,000.

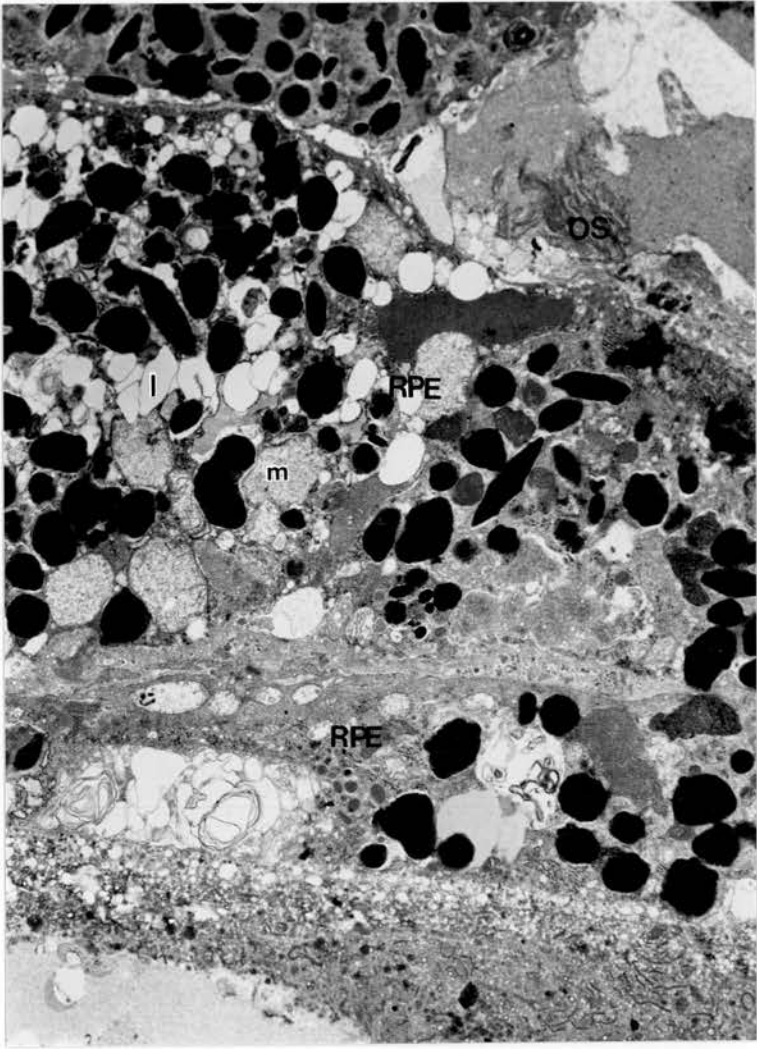


Figure II 8

Fig. II9A. Light micrograph of fovea of the left eye. There are pyknotic nuclei in the outer nuclear layer. The RPE is irregular and is focally detached from Bruch's membrane. Acellular material lies in the sub-RPE space. (Stain Mallory's Blue x 400).

B. Light micrograph of same area as in A showing irregular acellular material in sub-RPE space. (Stain Mallory's blue x 600).

Fig. II10A. Light micrograph of RPE detachment showing more advanced RPE and photoreceptor disorganisation than in Figure 8. (Stain Mallory's blue x 600).

B. Light micrograph of same area as in A showing a craggy deposit (d) closely associated with the RPE overlying the serous sub RPE material. (Stain Mallory's blue x 1,000).

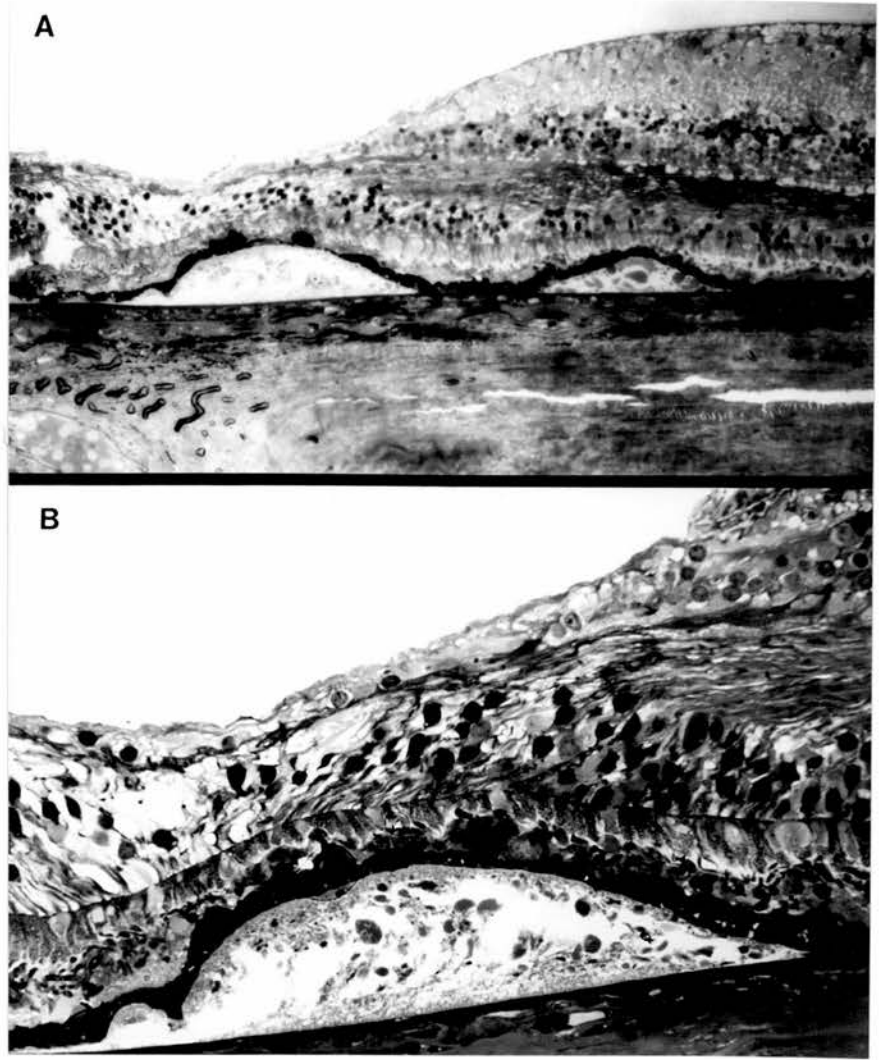


Figure II 9

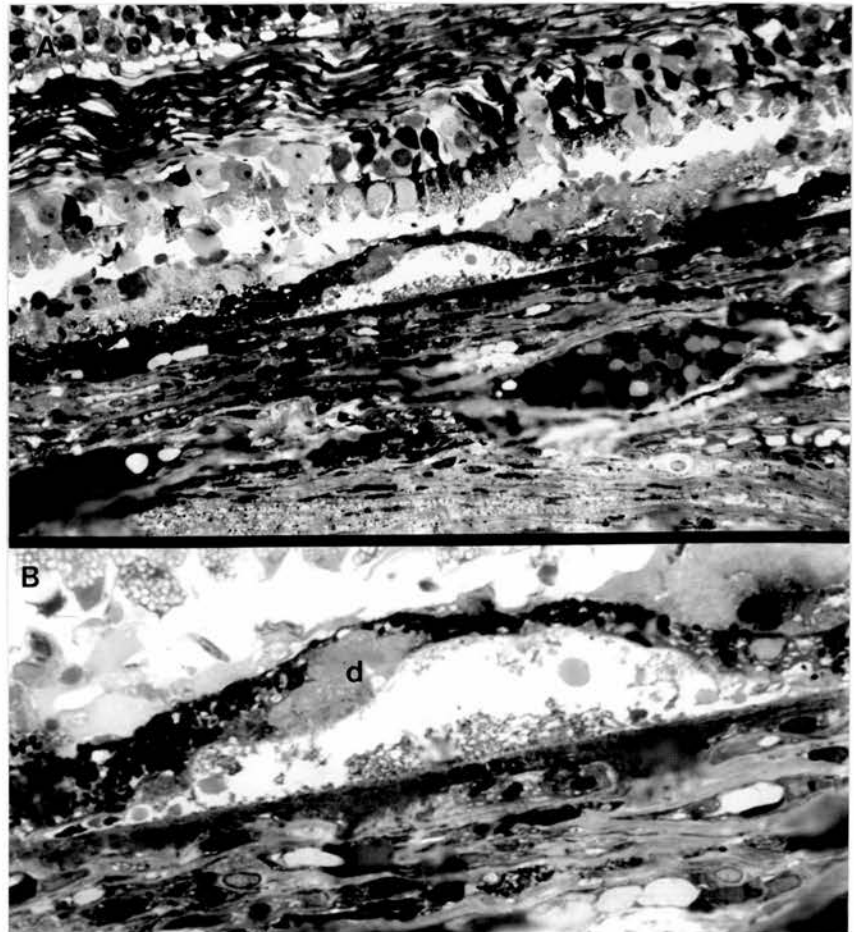


Figure II 10

material in the sub RPE space (Fig II 11,12,13) with vacuolation at its interface with the basal lamina of the RPE which was only focally identifiable, in other places being replaced by the vacuolated and banded basement membrane material in the RPE basal infoldings.

Bruch's membrane also showed advanced aging changes with vesicular and banded basement membrane deposition and curvilinear laminar basement membrane (Fig. II 14,15). The intercapillary distance was widened but the choriocapillaris was patent (Fig II 14).

The retinal pigment epithelial detachment in the right eye had a different appearance on light microscopy. There were pale homogenous globules of material in the sub RPE space, between layers of proliferated RPE, and in the sub-retinal space (Fig II 16).

Photoreceptor degeneration was more advanced than in the left eye.

Electron microscopically (Fig II 17,18) the globules had the same electron density as plasma, and were seen in association with red blood cells. The retinal pigment epithelium and Bruch's membrane showed similar changes to those seen in the left eye, but patent new vessels were present in the sub-RPE space (Fig II 19).

In sections adjacent to those showing sub-RPE vessels, collection of cells with spindle shaped nuclei and vacuolated cytoplasm were found (Fig II 20) which by

Fig. II11. Electron micrograph showing spectrum of degenerative changes in Bruch's membrane. These are vesicles banded collagen and curled membrane profiles (arrowheads) on both sides of the choriocapillaris (CC). A cell nucleus (n), probably of a pericyte (P), is seen lying between the basement membrane of the choriocapillaris and the remainder of Bruch's membrane. The intercapillary pillar is widened. There is detachment of the RPE with a faintly granular material filling the sub-RPE space, and banded collagen (d) in the basal infoldings of the RPE (x 12,000).

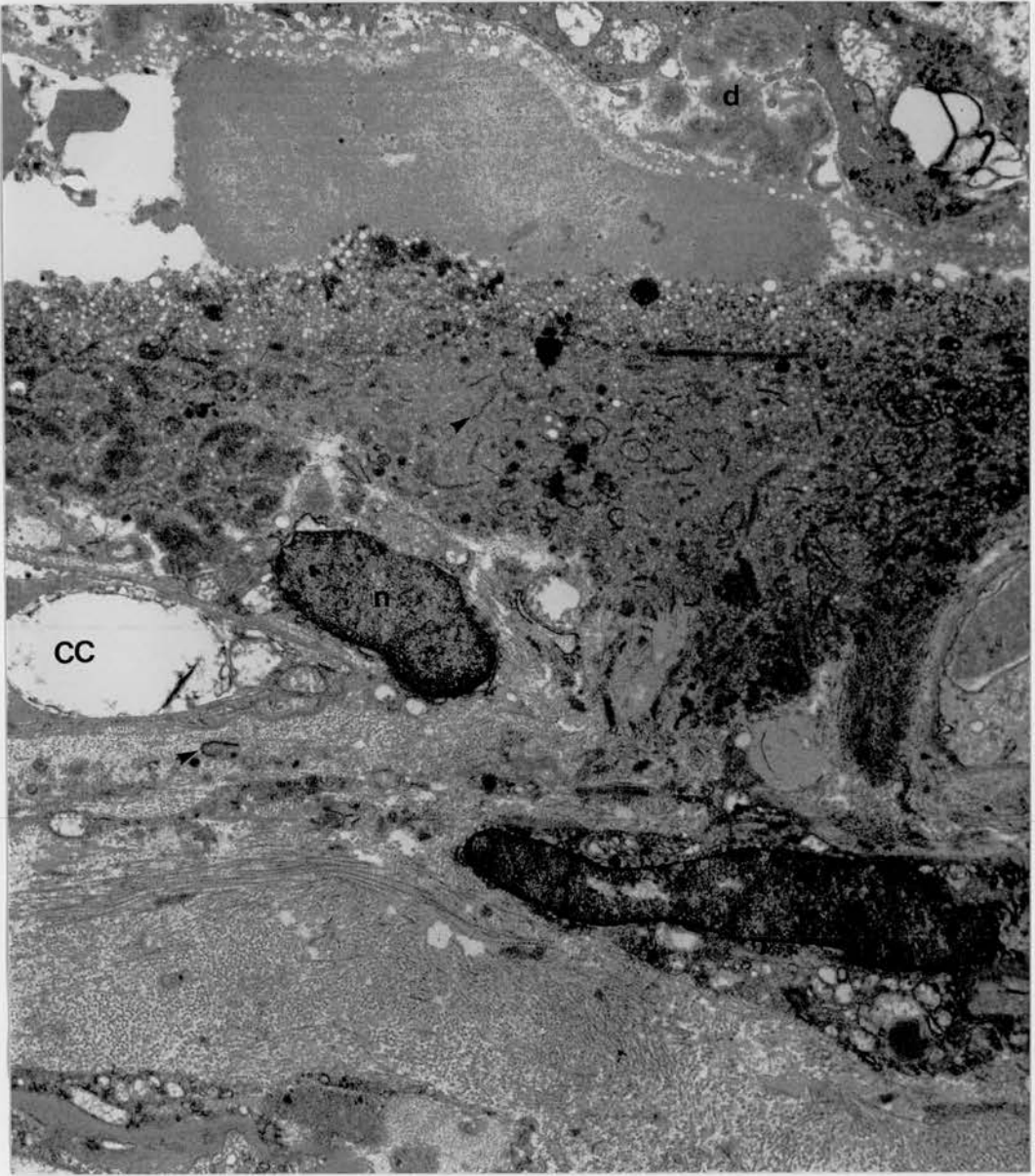


Figure II 11

Figure II₁₂. Higher power showing the sub RPE material which is finely granular and merges inseparably with the craggy deposit (d) between the RPE basal infoldings. Bruch's membrane contains numerous membrane-like profiles. (x 23,000).

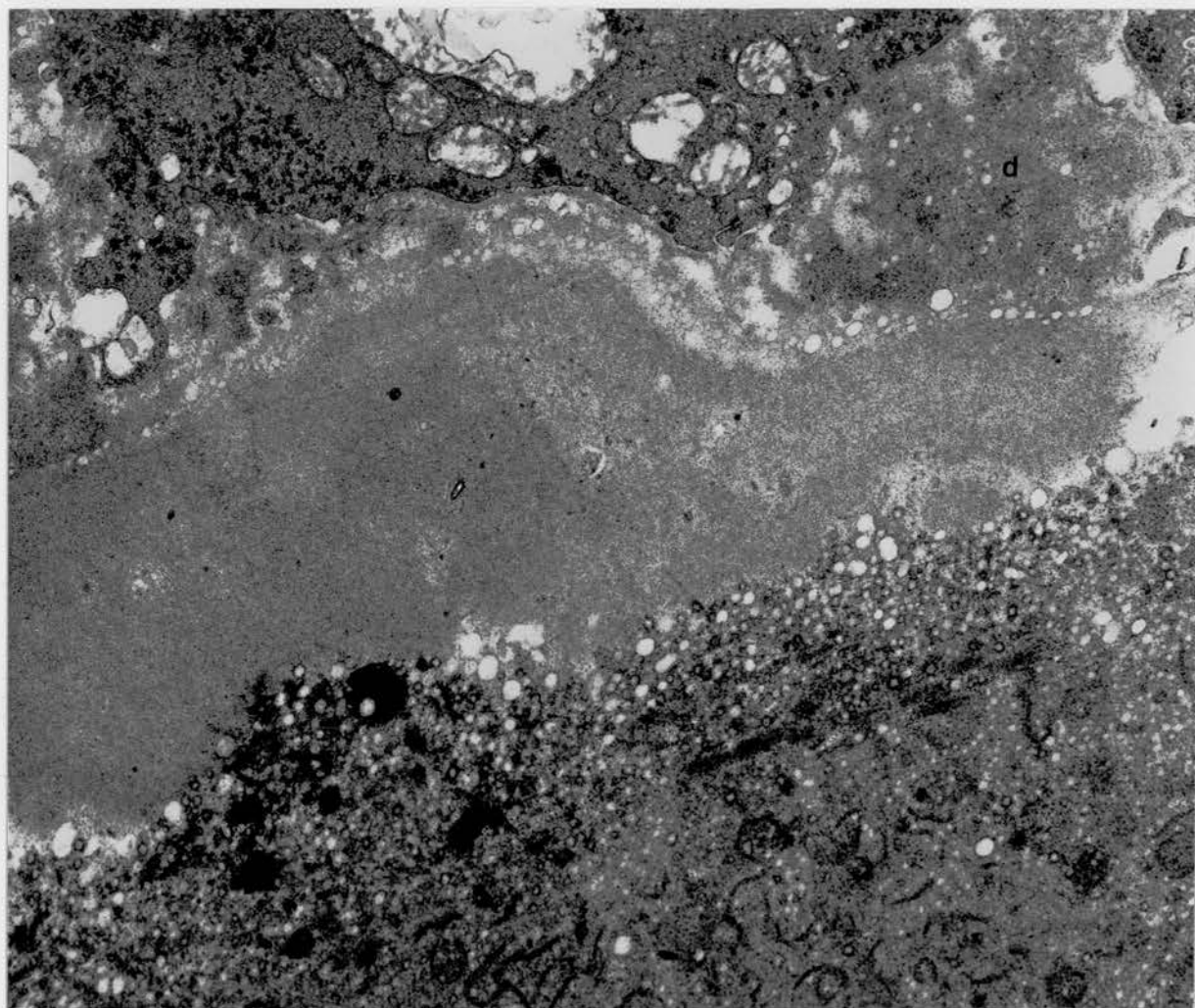


Figure II 12

Fig. II₁₃. Electron micrograph of the cell overlying the detachment shown in figure II₁₂. The RPE cell is grossly degenerate with lysed mitochondria (arrowhead) and condensed cytoplasm. An adjacent cell (RPE) is necrotic and free melanosomes (m) are present. Photoreceptor outer segments are absent. ($\times 12,000$).

Fig. II₁₄. Electron micrograph showing extensive deposit in Bruch's membrane, characterised by vesicular structures, curvilinear structures and some wide spacing collagen, on both sides of the choriocapillaris (CC). Note the extensive craggy deposit ($\times 7,000$).

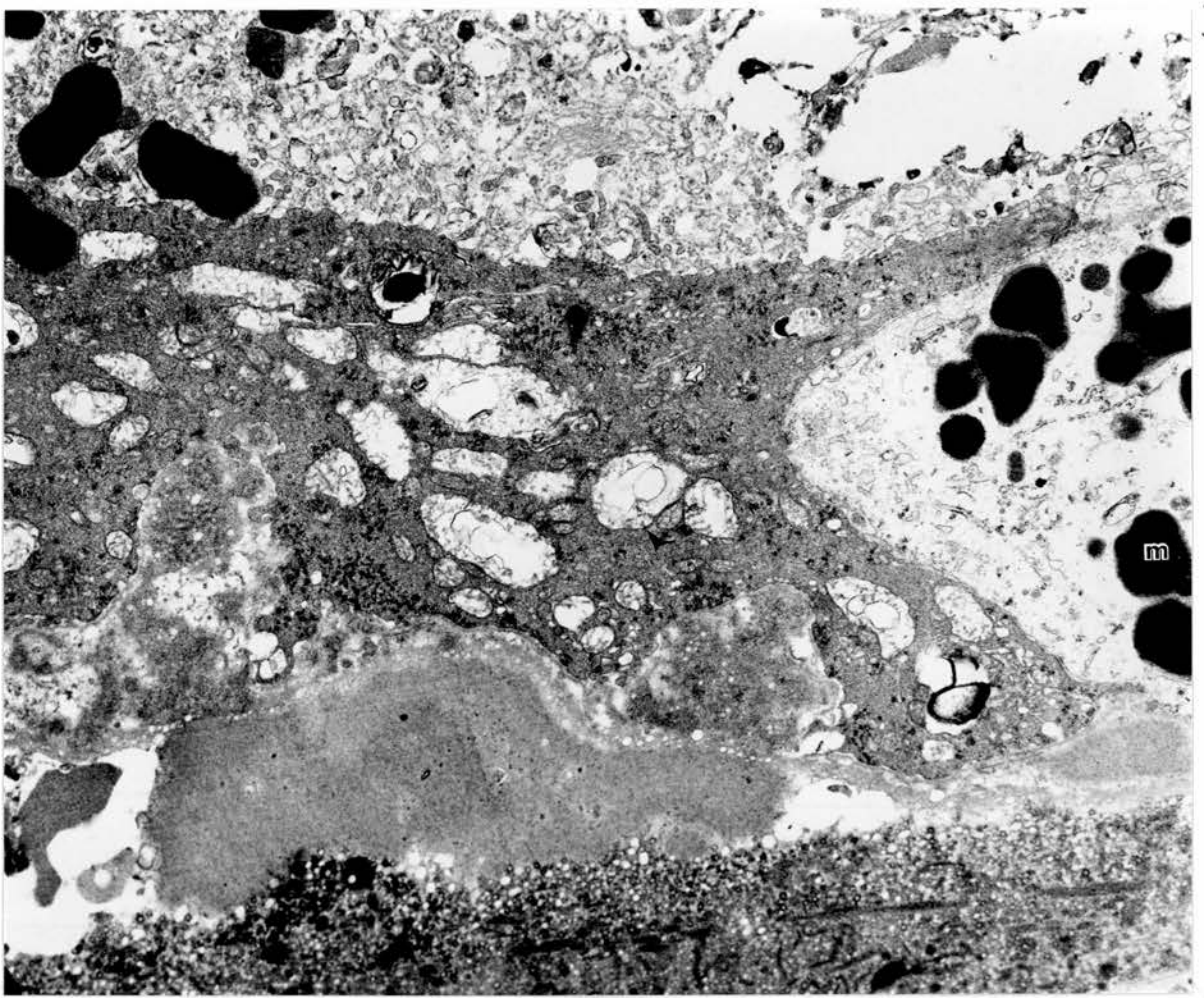


Figure II 13

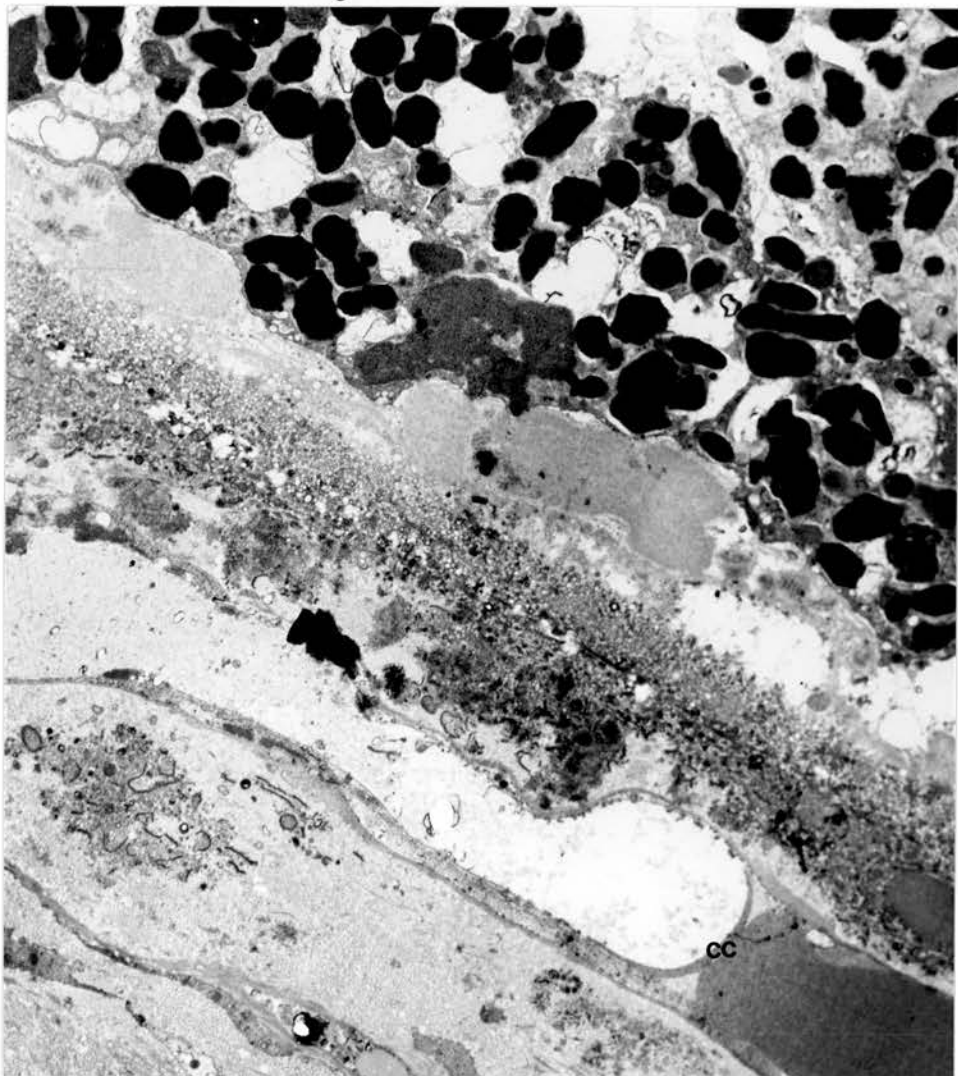


Figure II 14

Fig. II₁₅. Electron micrograph showing higher power detail of age related deposit. Note the appearance of coated vesicles (arrowheads). Between Bruch's membrane (BM) and the RPE (RPE) are a number of endothelial cells (E) $\times 23,000$.



Figure II 15

Fig. II16. Light micrographs of the macular area of the right eye. A, B. Globular material (g) is present under the RPE and between layers of proliferated RPE. The choriocapillaris is sclerosed and there is marked loss of photoreceptors and nuclei in the outer plexiform layer. Larger choroidal vessels are patent (Mallory's blue stain x 600). C. Globular material (g) is present in the subretinal space with marked distortion and loss of photoreceptor elements and nuclei. The pigment epithelium is separated from Bruch's membrane by a thick deposit of basement membrane material (d). The choriocapillaris is sclerosed. (x 600).

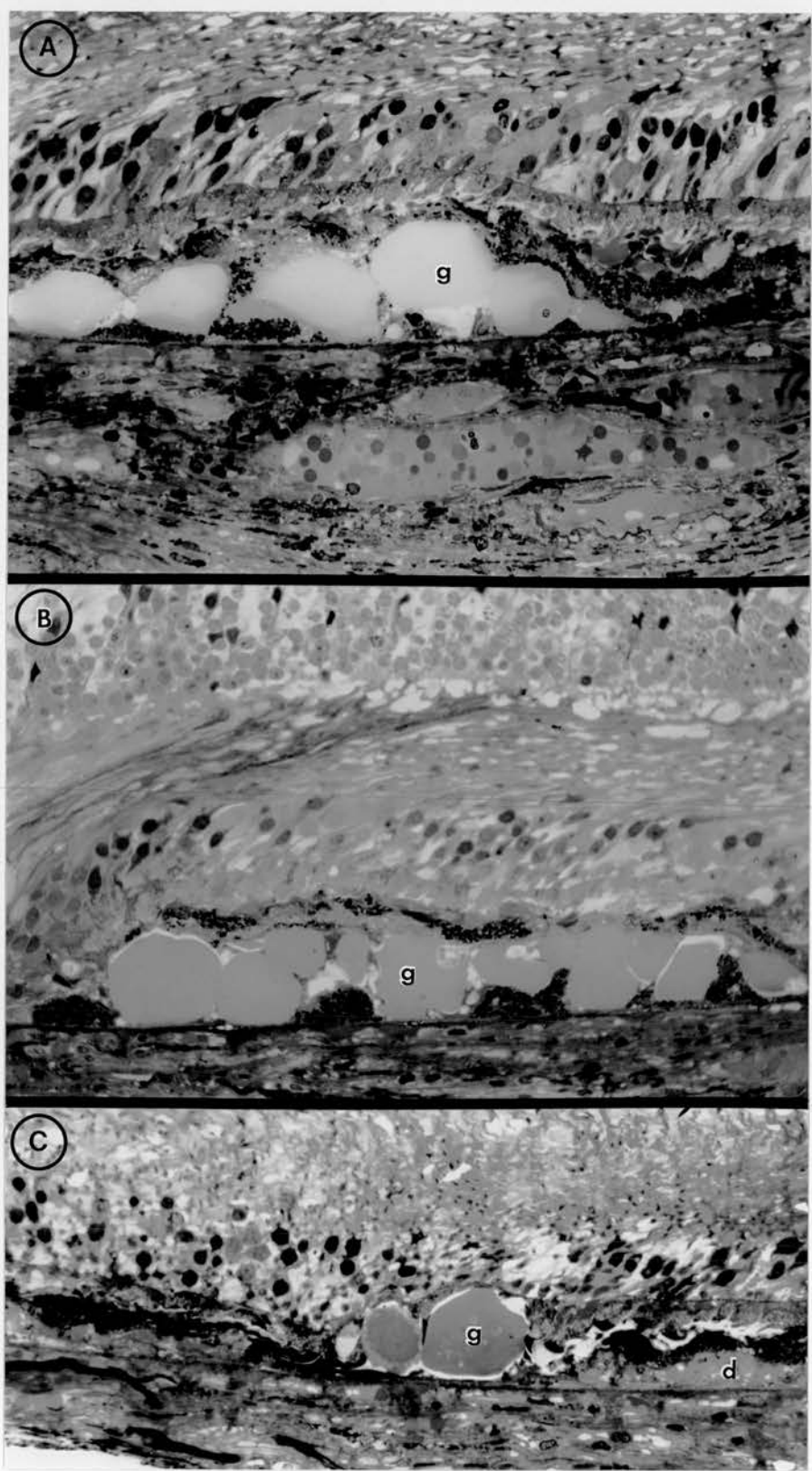


Figure II 16

Fig. II₁₇. Electron micrograph of the macular area of the right eye showing globule (g) of moderately electron dense material with peripheral vesicle formation in the subretinal space. (IS - inner segment, rbc - red blood cell, BM - Bruch's membrane. (x 5,000).

Fig. II₁₈. Electron micrograph showing globular deposit (g) separated from Bruch's membrane by an attenuated RPE (RPE) cell. (x 5,000)

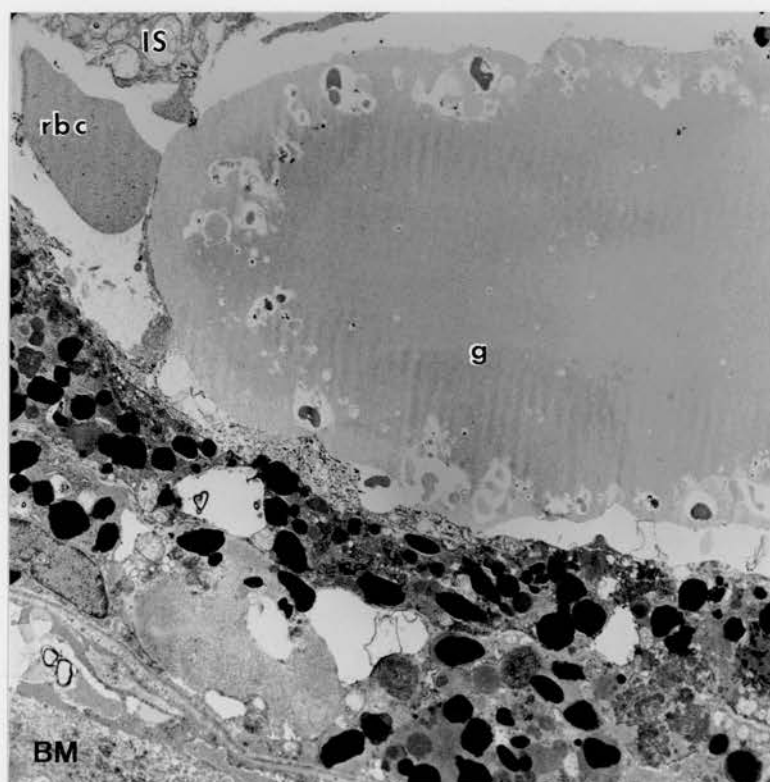


Figure II 17

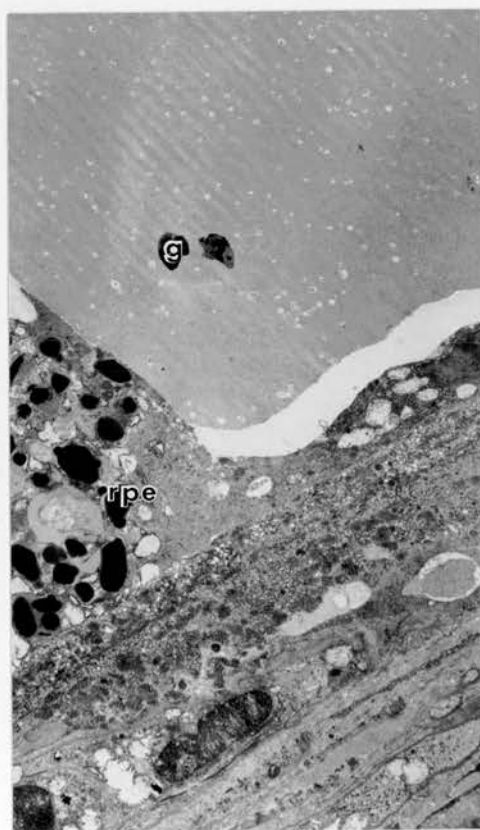


Figure II 18

Fig. II₁₉. Adjacent area to Fig II₁₇ and Fig II₁₈.
A sub-RPE new vessel (NV) is present (OS-outer segment, E-endothelial cell). (x 5,000).

Fig. II₂₀. A light micrograph showing sub-RPE cells. B. Light micrograph showing multilayered sub-RPE cells (stain Mallory's blue x 1,000).

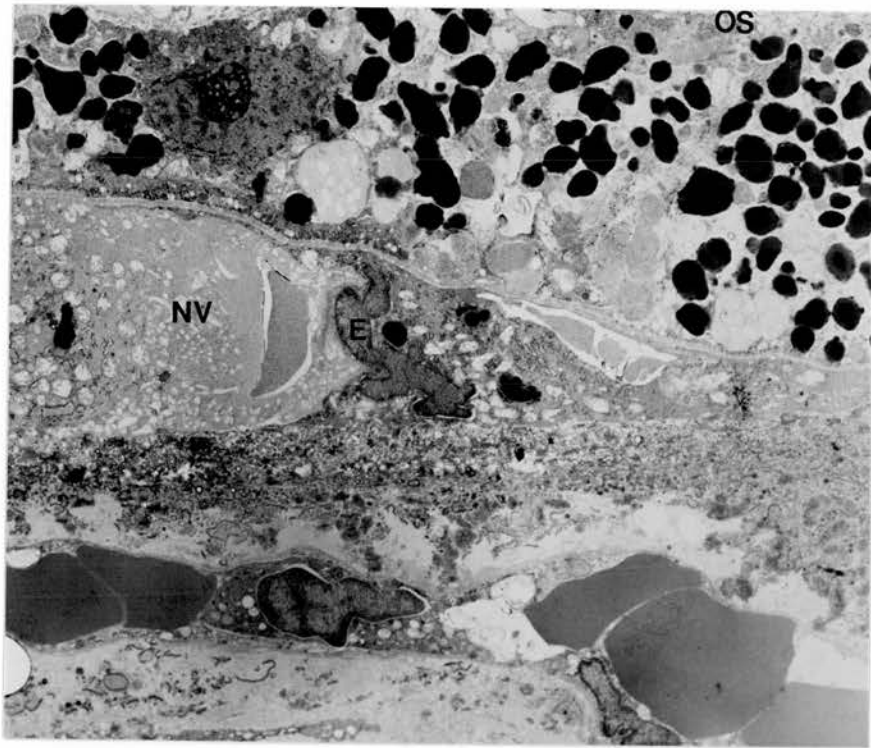


Figure
II 19

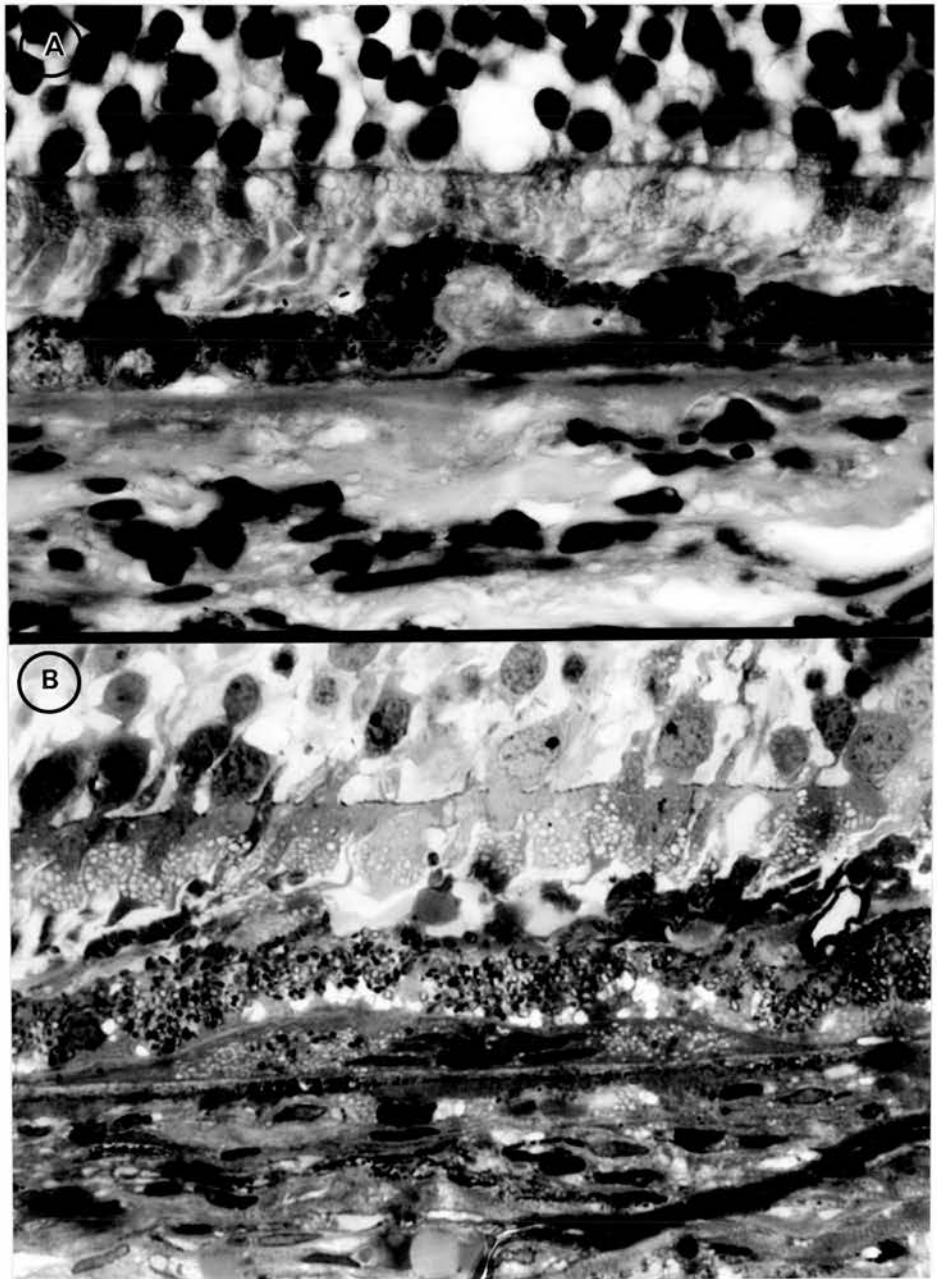


Figure
II 20

electron microscopy were identified as endothelial cells, having scanty electron dense cytoplasm containing numerous mitochondria filaments and rough endoplasmic reticulin (Fig II 21-26). They were closely associated with the mixed basement membrane deposits in the RPE basal infoldings. Some of these cells contained large variably dense membrane bound cytoplasmic organelles possibly lysosomes, and also coated vesicles.

The choriocapillaris was largely occluded in the left eye (Fig II 28). The endothelial cells were greatly swollen, and the linear basal lamina was thickened and enveloped numerous watery cytoplasmic processes containing filaments, mitochondria and pinocytotic vesicles. Cell nuclei were found external to the basal lamina of the choriocapillaris (Fig II 28,29) in both eyes and were identified as pericytic on the basis of their location. Elsewhere, there was a laminated arrangement of thick basement membrane investing cytoplasmic processes which contained few organelles other than mitochondria, filaments and numerous pinocytotic vesicles (Fig II 29B,30,31). These cells are not be confused with endothelial cells of collapsed capillaris (Fig II 30A) which are totally enveloped in thick basement membrane and are seen in relation to slit-like lumina. In places it appeared that endothelial cells were leaving the choriocapillaris moving towards Bruch's membrane (Fig 30 A). Cells containing numerous pigment granules and phagosomes were present in the subretinal space and in the sub RPE space (Fig II 32,33). The various changes are shown diagrammatically in Fig II 34.

Fig. II₂₁. Electron micrograph of a sub-RPE cell with a spindle-shaped nucleus, and dense cytoplasm underlying craggy deposit (d) (x 7,000).

Fig. II₂₂. Electron micrograph showing sub-RPE cell with mitochondria (m), rough endoplasmic reticulum (er), and coated vesicles (v). (x 16,000).

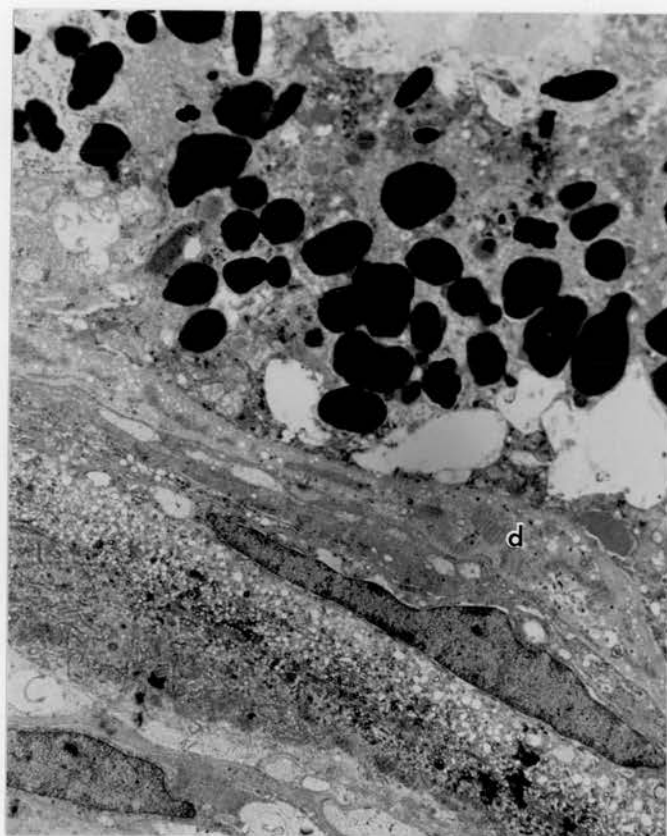


Figure II 21

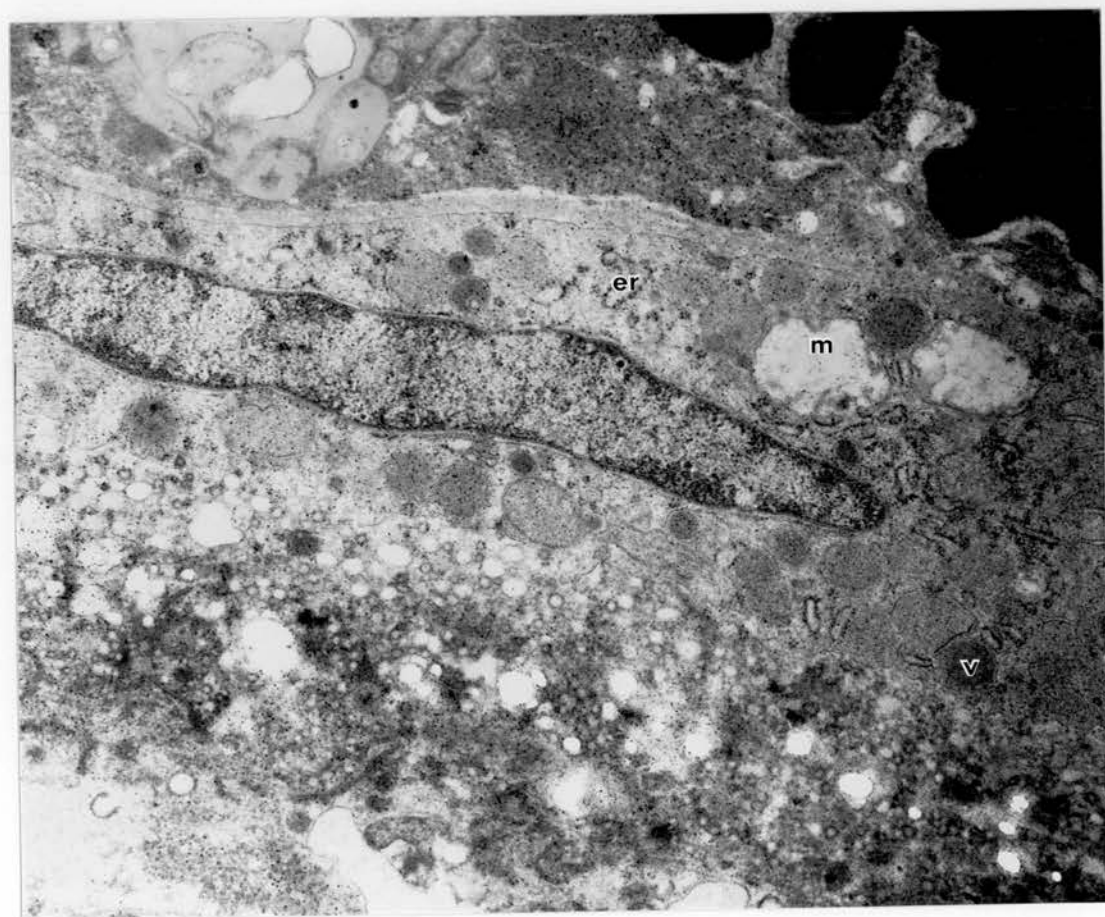


Figure II 22

Fig. II₂₃. Electron micrograph of a sub-RPE cell shown at lower power in Fig. II₂₁. Note that it does not have an enveloping basement membrane. Cytoplasm from a number of cells (E) is present in a multilaminar arrangement. Cytoplasmic filaments (arrowheads) are prominent. Note copious craggy deposit (d). (x 23,000).

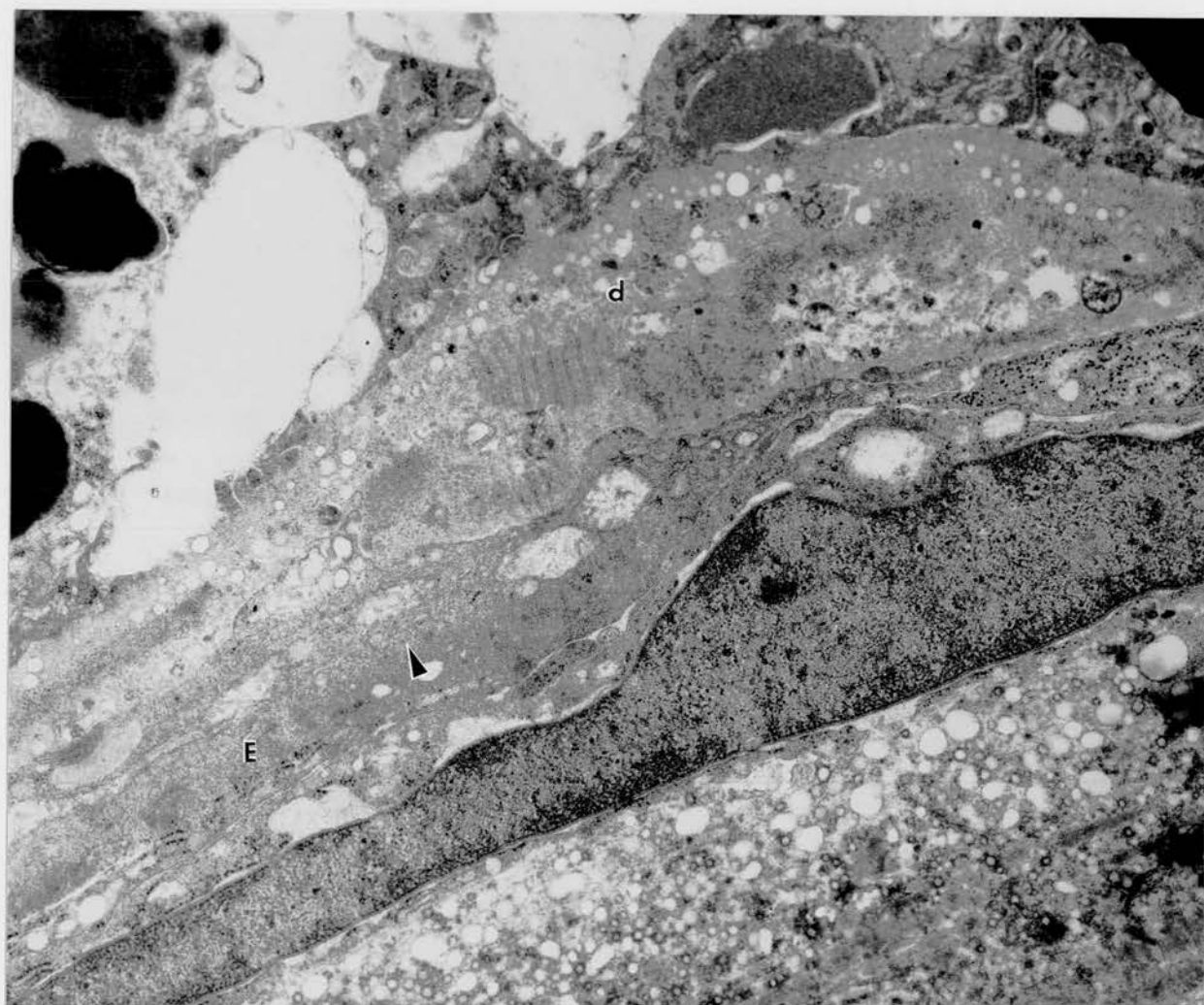


Figure II 23

Fig II₂₄. Electron micrograph showing several cell processes between the choriocapillary endothelium (CC) and detachment (d). The innermost (a), contains numerous filaments (arrowhead) and is probably endothelial, by comparison with fig. II 23. The next (b), contains pigment granules as well as filaments. Bruch's membrane is greatly thickened. On the outer aspect processes (c), with filaments and lysed mitochondria are seen. The latter two types are probably also endothelial or pericytic (x 23,000).

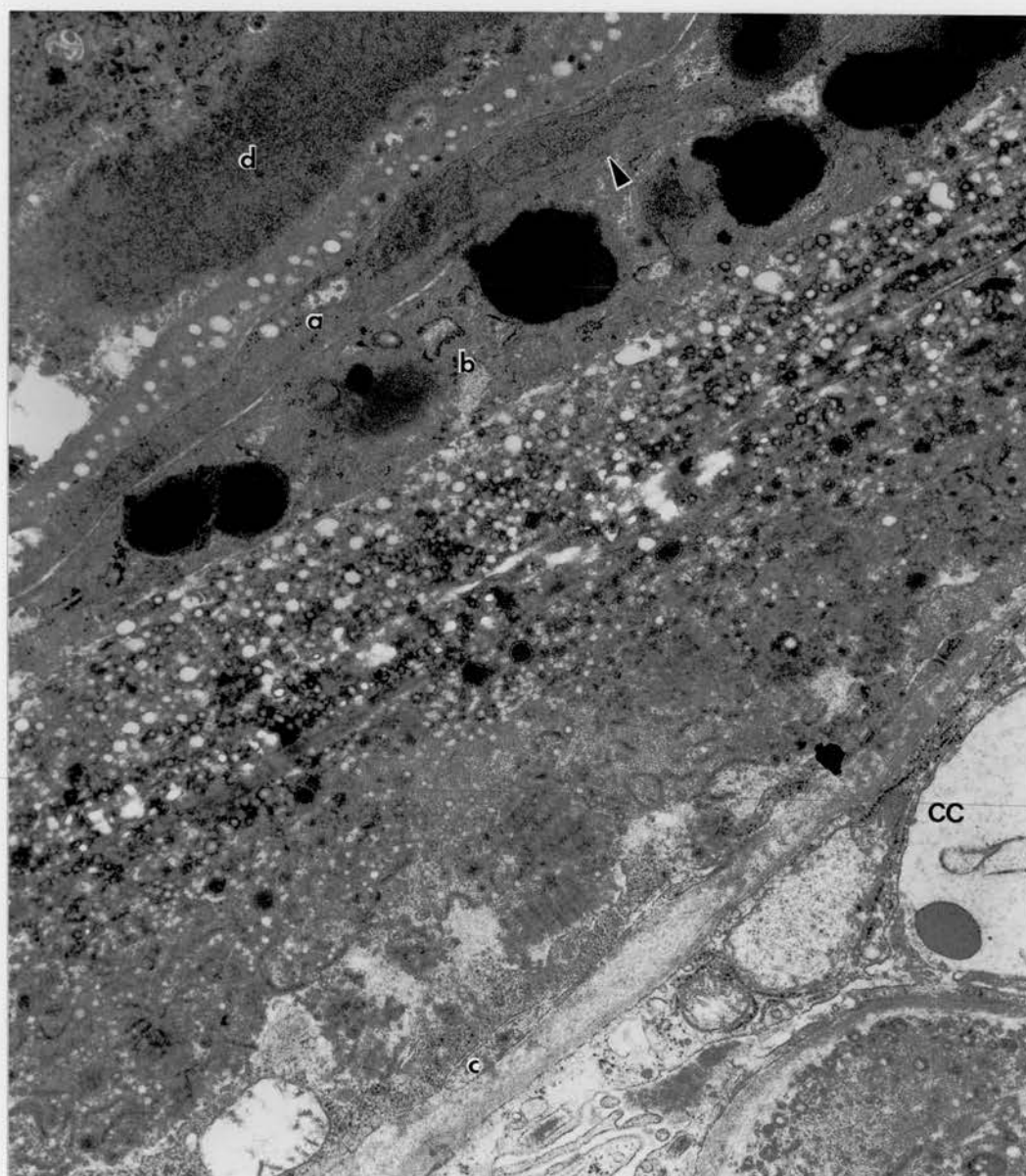


Figure II 24

Fig. II₂₅. Electron micrograph of a cell lying in the sub RPE space characterised by a spindle shaped nucleus, mitochondria, lysosomes, coated vesicles and rough endoplasmic reticulum. (x 16,000).

Fig. II₂₆. Electron micrograph. There is craggy deposit (d) under the RPE. Between the RPE and the inner collagen layer of Bruch's membrane is a degenerate cell with dense cytoplasm, lysed mitochondria and numerous coated vesicles. Bruch's membrane shows extensive ageing change. Choriocapillary endothelial cells (E) are identifiable but the lumen is obliterated. (x 12,000).

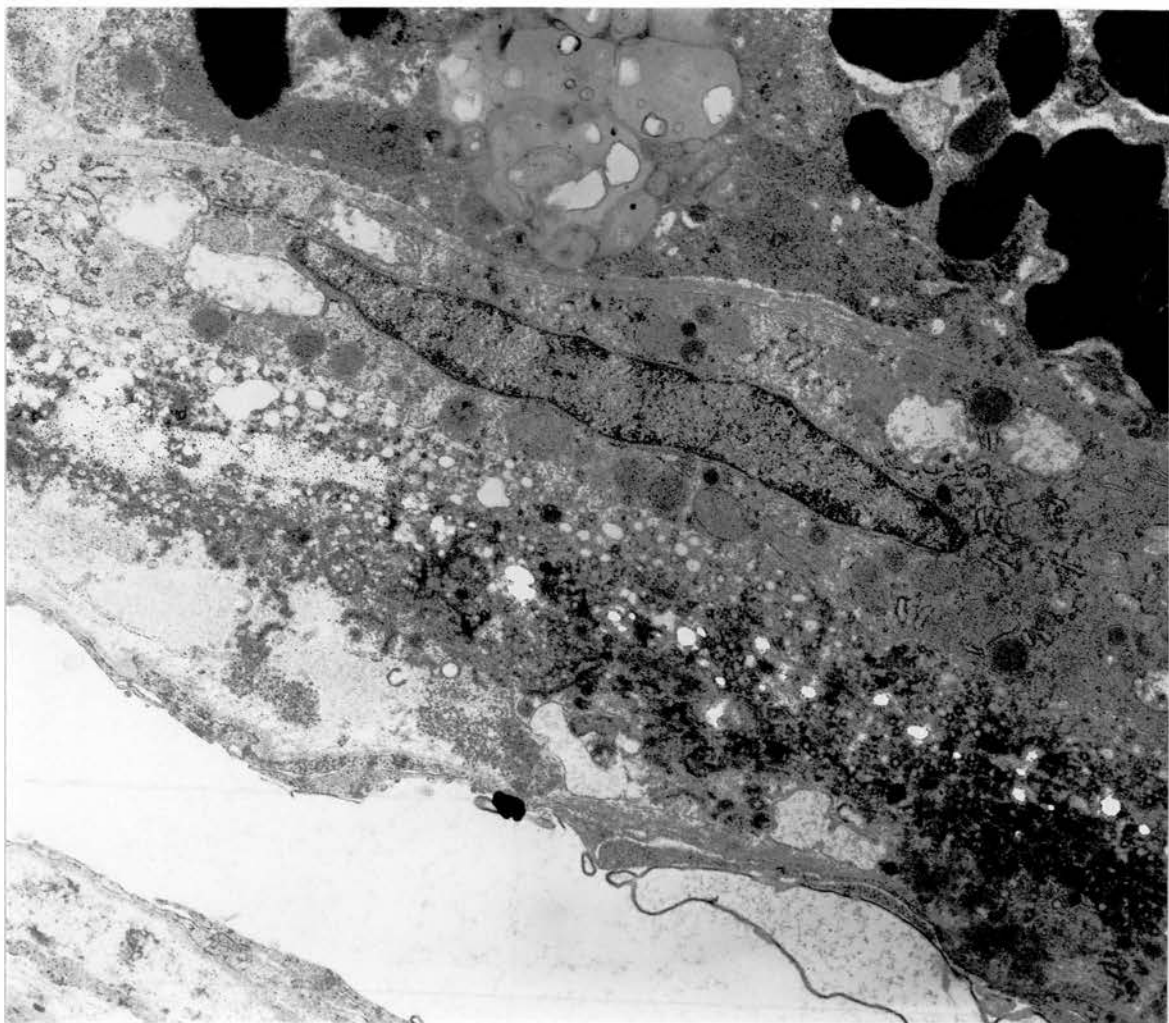


Figure II 25

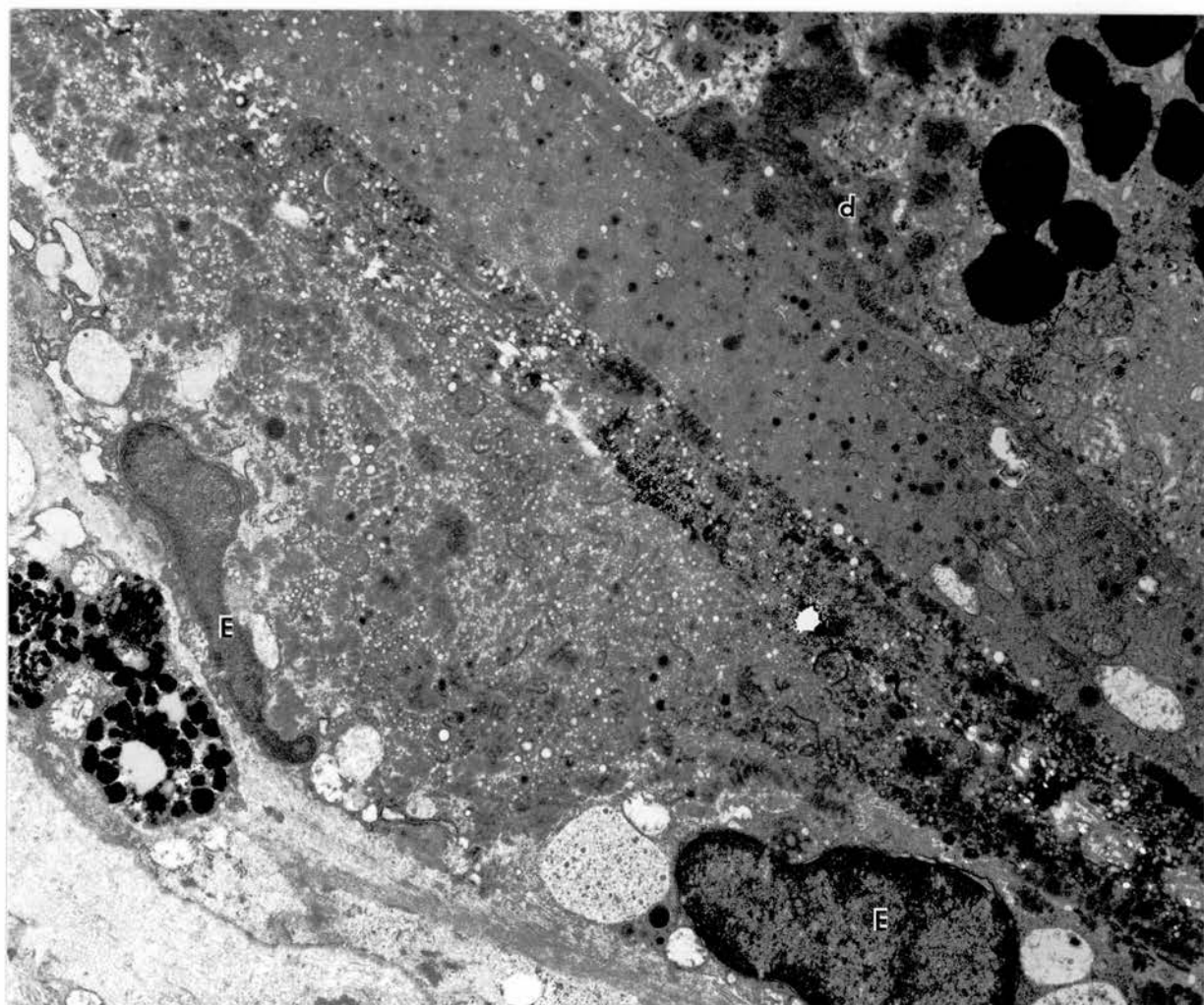


Figure II 26

Fig. II_{27A}. Electron micrograph of clumps of laminated membrane material first shown in Fig. II_{7A}. It is not possible to determine whether it lies intra or extra cellularly in relation to the RPE. (x 12,000).

B. Electron micrograph of same material as in A. In this instance it is in the sub RPE space and shows a trilaminar arrangement in one plane and circular profiles in the other. (x 47,000).

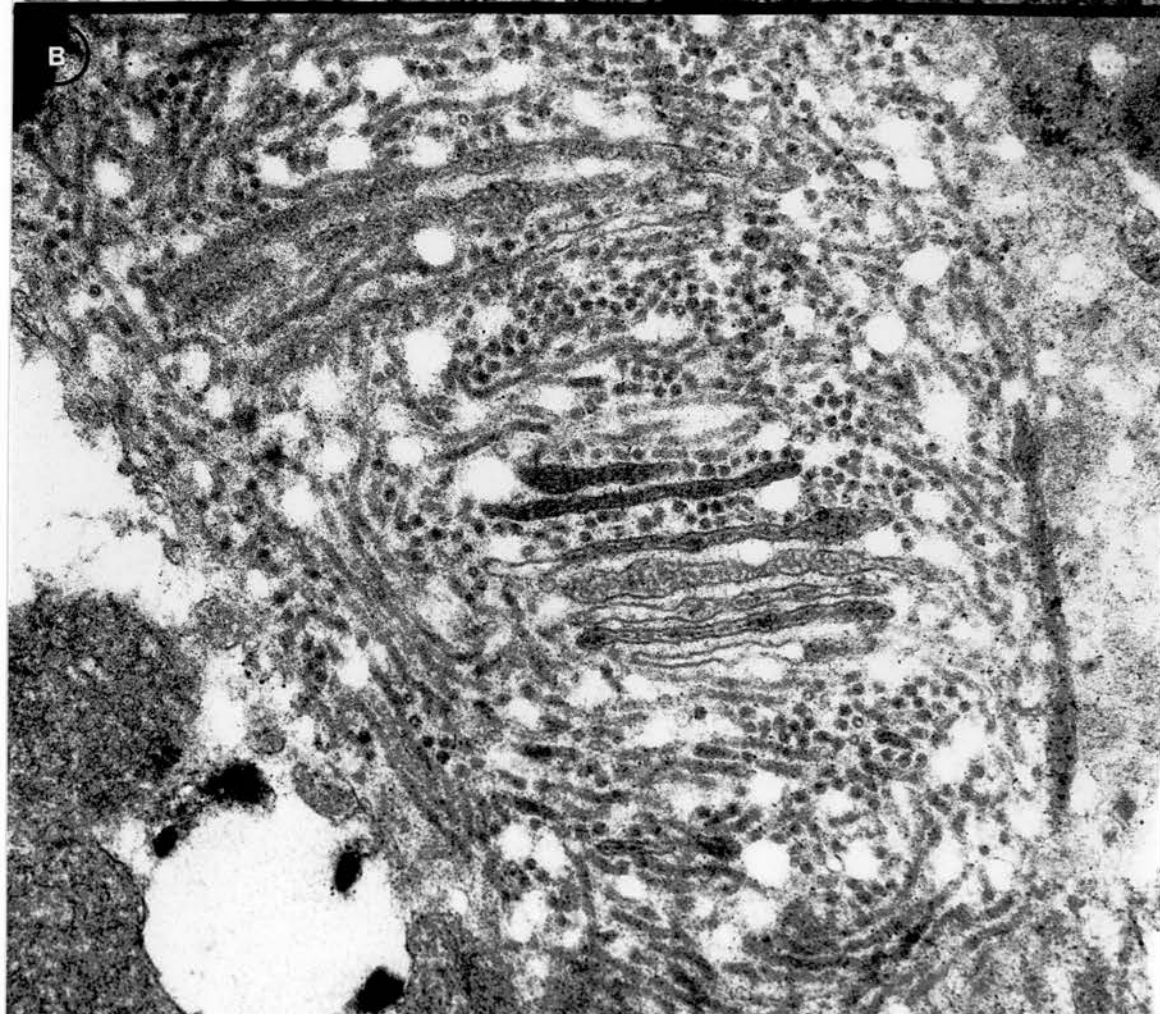
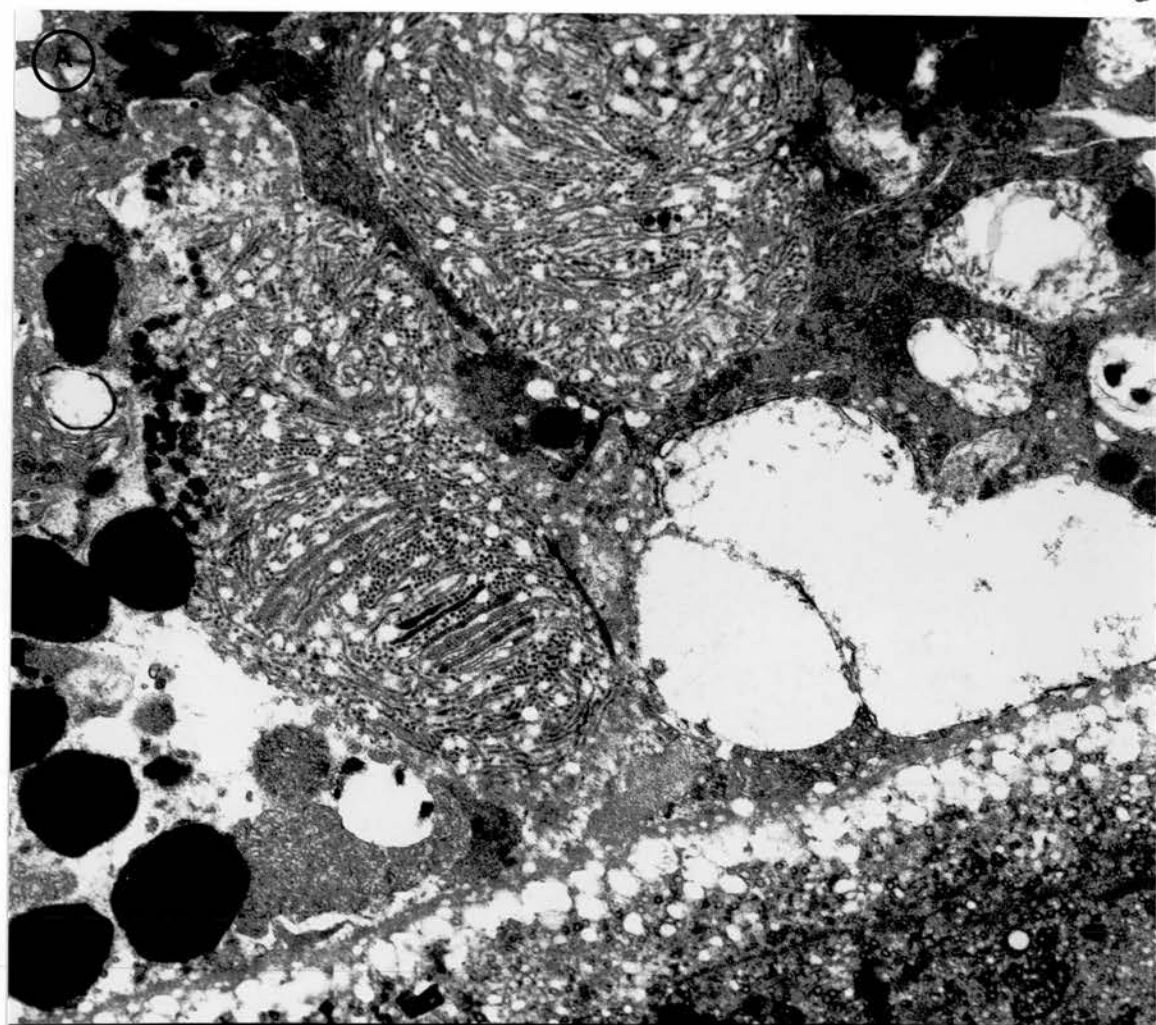


Figure II 27

Fig. II28. Electron micrograph of a choriocapillary surrounded by cell processes (p) invested in basement membrane. The lumen (l) is greatly narrowed. A cell with an oval nucleus (n) and dense cytoplasm containing numerous mitochondria lies internal to the capillary. Bruch's membrane is thickened and contains banded collagen (d) and membrane profiles ($\times 7,000$).

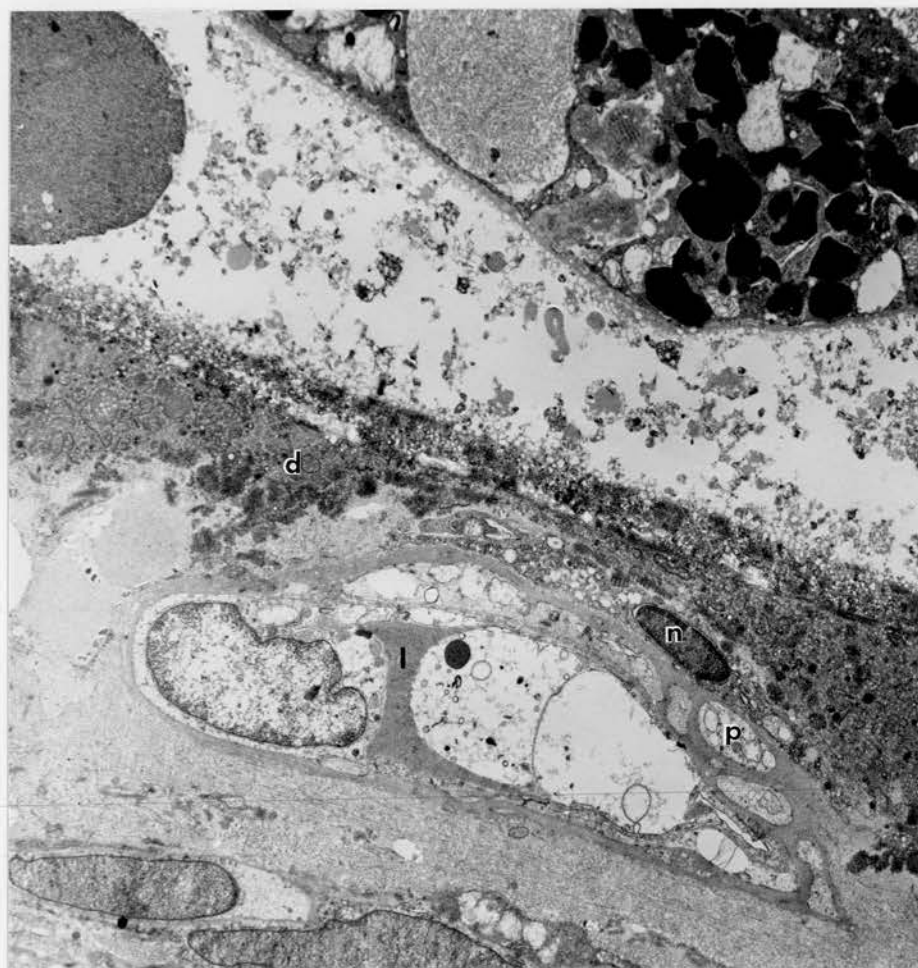


Figure II 28

Fig. II29A. Electron micrograph showing cell on inner aspect of choriocapillary in Fig. II28 at high power. Note the pericyte process (P) enveloped in thick basement membrane. (x 23,000).

B. Electron micrograph of Bruch's membrane showing watery cytoplasmic process with filaments (small arrow) and pinocytotic vesicles (arrowhead) (x 23,000).

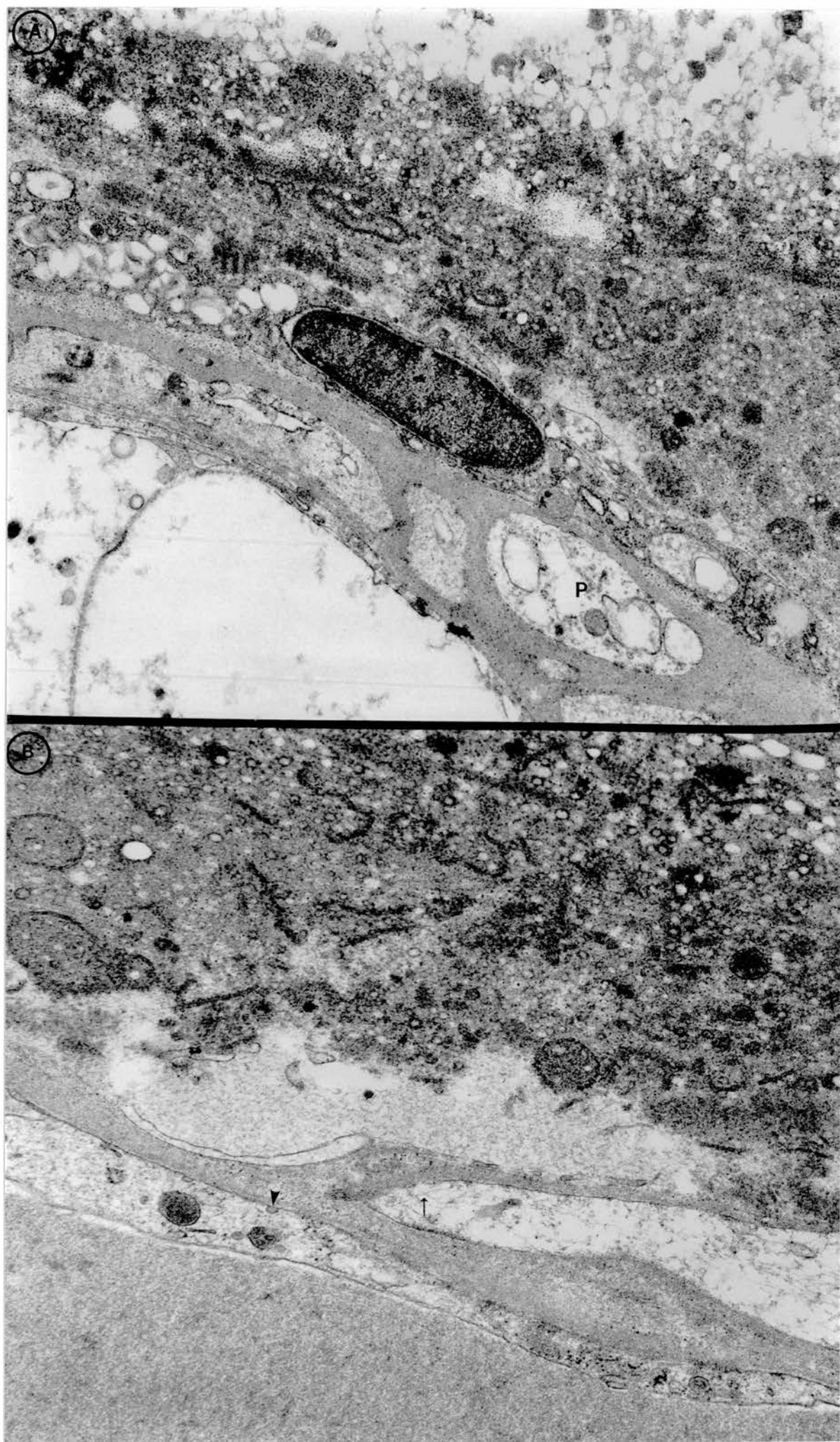


Figure II 29

Fig. II30A. Electron micrograph of endothelial cell (E) of collapsed capillary surrounded by thick basement membrane, which also envelops a watery pericyte process (P) and is surrounding a red blood cell (RBC). The endothelial cell cytoplasm contains numerous swollen mitochondria (m) ($\times 4,000$)

B. Electron micrograph of an endothelial cell (E) which is unusually elongated. It is partially enveloped in basement membrane, but does not appear to be closely related to the choriocapillary lumen (L). Other similar cells without contact with the lumen are also present. ($\times 4,000$).

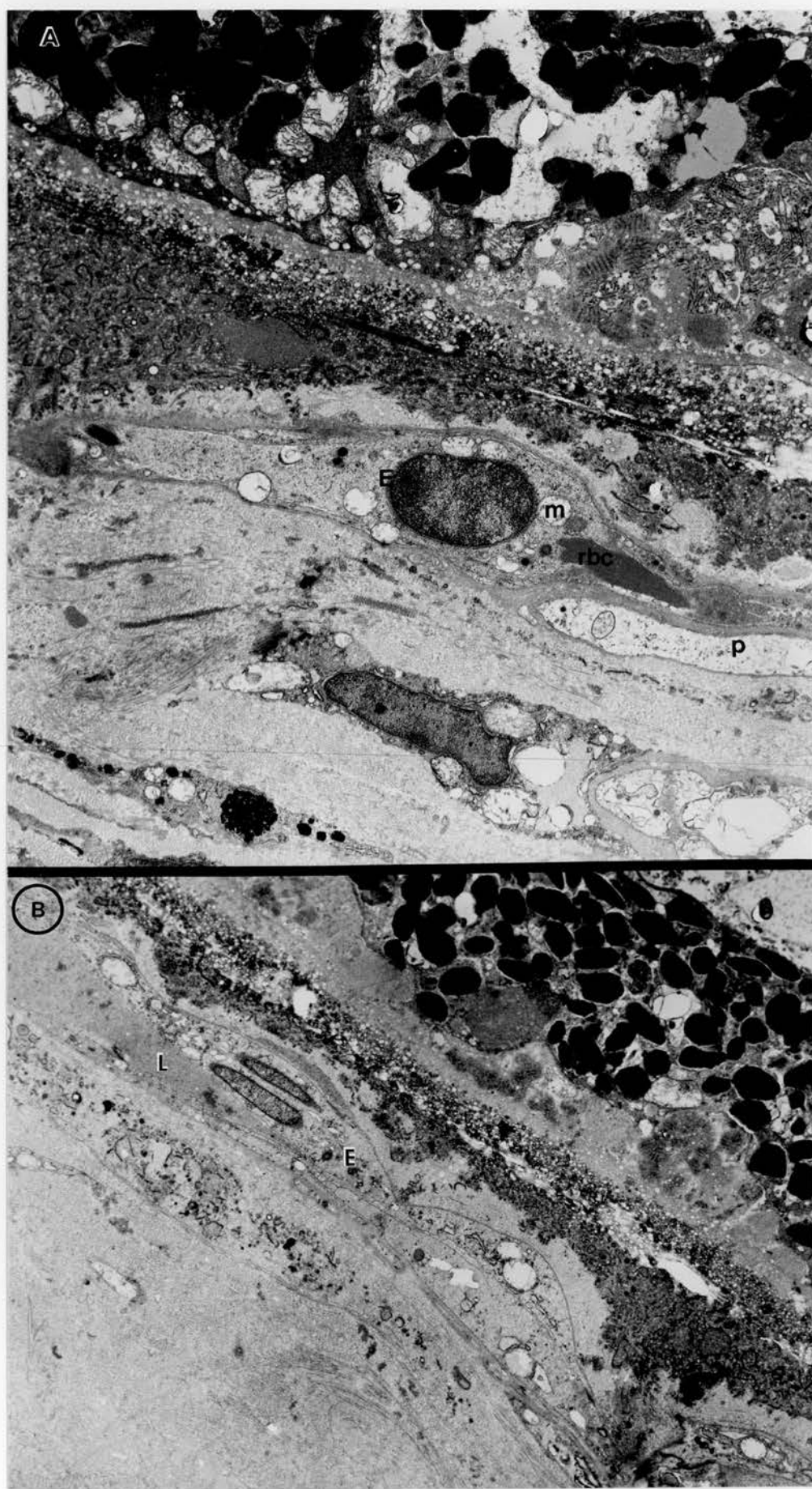


Figure II 30

Fig. II31A. Electron micrograph from an area in which the choriocapillaris has been obliterated. Cytoplasmic processes (p) lie in a laminated arrangement between layers of basement membrane. Organelles include mitochondria (m) and pinocytotic vesicles (arrowhead) (x 23,000).

B. Electron micrograph showing processes of cells in Fig. II298 with cytoplasmic filaments and pinocytotic vesicles (arrowhead). (x 47,000).

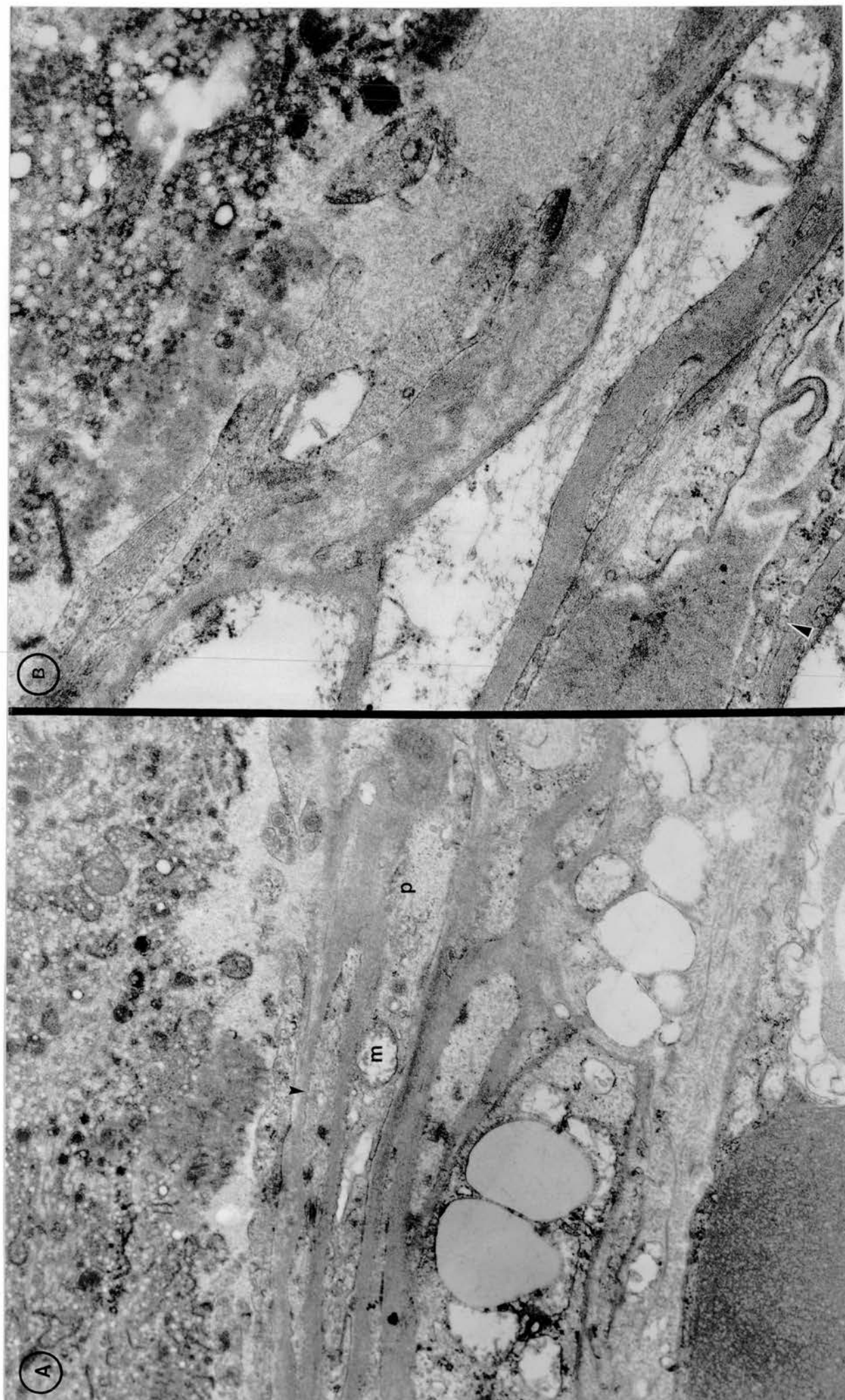


Figure II 31

Fig. II₃₂. Electron micrograph of a rounded pigment containing cell in the subretinal space, containing phagocytosed outer segments (P) and an indented nucleus (n). The surrounding RPE is degenerate. BM Bruch's membrane (x 7,000).

Fig. II₃₃. Electron micrograph of a pigmented cell (m) in the sub RPE space (x 7,000).

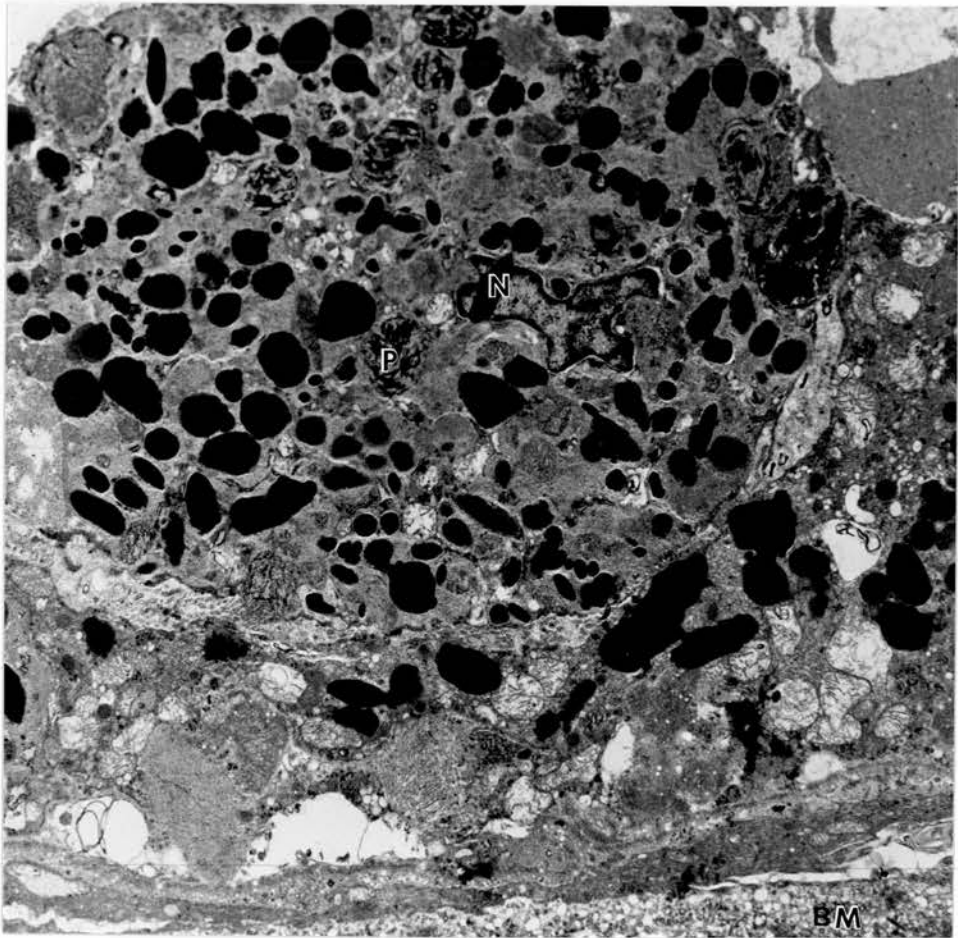


Figure II 32

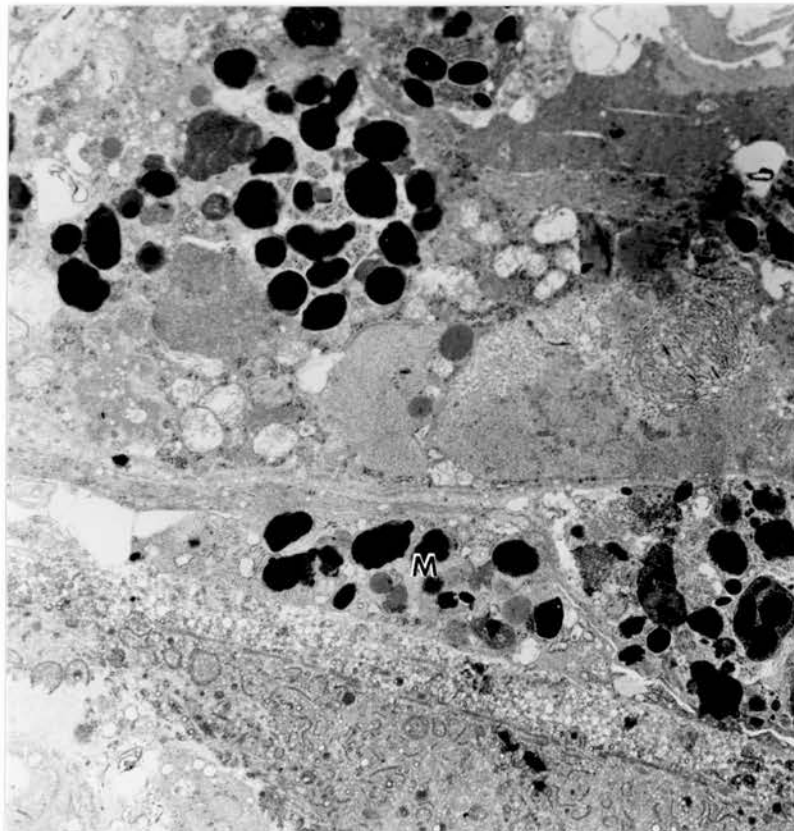
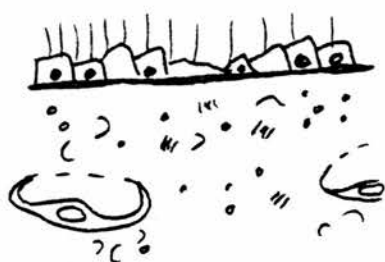


Figure II 33

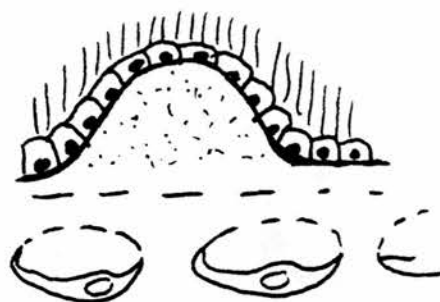
Fig. II34. Diagram representing features of ageing
and degenerative change.

1



Ageing RPE, Bruch's
membrane and
choriocapillaris

2



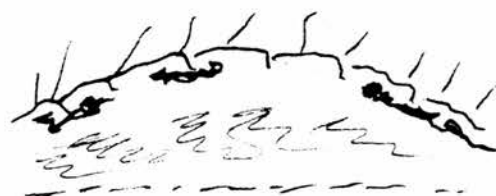
"Typical" druse

3



Excessive basement
membrane

4



Avascular RPE
detachment

5



Neo vascularisation

6



Exudative RPE
detachment

Discussion

The findings in the two eyes were compared and contrasted. Both showed the changes of age related macular degeneration, with advanced RPE degeneration and RPE detachment. In the phakic eye, RPE degeneration was associated with reactive change and excessive production of banded and vesicular basement membrane, as defined by Fine and Yanoff (320). The membranous material in the RPE, illustrated in Fig. II 7A,27 was not characterised, but it may be a product of outer segment phagocytosis with incomplete digestion.

In the left eye the deposit was sufficiently extensive to give the appearance of RPE detachment while in the right there was sub RPE neovascularisation and exudation of plasma into the sub RPE and subretinal space.

Clinically, RPE detachment may be seen in association with choroidal inflammation, ischaemia or choroidal tumours, as well as being an idiopathic finding in some young patients with or without associated neurosensory retinal detachment (404). In elderly patients it is considered to be most often due to exudation from sub RPE new vessels which are recognised by fluorescein angiography (79A,404,410). However, in 10% of cases no neovascular frond is seen (320) although, the risk of developing sub RPE neovascularisation is great.

The aging changes in the choriocapillaris, Bruch's membrane and RPE were reviewed in Chapter 1 and are confirmed in this study.

The detachment of the RPE in the left eye may have occurred as a result of disturbance of the osmotic gradient between the sub RPE space and the choriocapillaris induced by the extensive basement membrane production by the RPE. This is the process termed "softening" by Sarkis (997) and now recognised by other authors (408,451,604,1040). It is recognised that aging changes in Bruch's membrane and the RPE progress to accumulation of basal linear deposit, then RPE detachment, sub RPE neovascularisation and a disciform process. This condition is separate from "typical" drusen which are situated in the inner collagenous zone of Bruch's membrane, with the basal lamina of the RPE intact over them.

The stimulus for neovascularisation is unknown. Suggested factors are choroidal ischaemia (618,1157) inner retinal ischaemia (636) and inflammation in response to the abnormal deposit (609,900,901).

Progression of age related macular degeneration following cataract surgery has previously been documented (88). It may be that the low grade uveitis following anterior segment surgery provoked the neovascular response, since it is known that macrophages stimulate endothelial proliferation (926). However, in this case no inflammatory cells were seen, but the underlying

pathology may be altered by the therapeutic use of steroids.

The cells present in the sub RPE space and Bruch's membrane are identified as endothelial cells, with spindle shaped nuclei, electron dense cytoplasm, mitochondria, rough endoplasmic reticulin and filaments. Coated vesicles were a prominent feature, although their role in cell metabolism is not known (209). Pericytes were also seen, characterised by the basement membrane which partially envelops them, watery cytoplasm numerous pinocytotic vesicles and filaments. These two cell types are known to participate in the neovascularisation process (33).

Clinicopathological correlation of the various histopathologically defined lesions can be made. Typical drusen correlate clinically with the small discrete white spots in the equatorial and posterior polar fundus, which are hyperfluorescence on fluorescein angiography. RPE degeneration with excessive basement membrane production correlates with mottling of the pigmentation at the macula, which on fluorescein angiography was hyperfluorescent but less intensely than the "typical" drusen, and had a shaggy outline. Patchy hypopigmentation on fundus examination, with corresponding area of hyperfluorescence on fluorescein angiography correlates with progression to avascular RPE detachment. The hazy and slightly elevated look to the macula which on fluorescein angiography showed intense leakage most noticeable in the late phase of the

angiogram was attributable to neovascular RPE detachment. The hypofluorescent patch with irregular borders on fluorescein angiography is well recognised as sub RPE neovascularisation.

The literature and the terminology of deposits in relation to the RPE-Bruch's membrane-choriocapillaris complex was reviewed in Chapter I. The term "drusen" is currently being used for a variety of lesions and both its clinical and its histopathological definitions are very loose. Terms used to differentiate different types of drusen are not universally accepted. These include typical drusen (412A), hard drusen (451,997), cuticular drusen (408), soft drusen (997), basal laminar drusen (412A), basal laminar deposits (714A,994), diffuse drusen (604), and confluent drusen (451). The dividing line between these and retinal pigment epithelial detachment (79A) is imprecise, and the relationship to the other yellowish or "vitelliform" (403,412A,747A,1051A) lesions is unclear. In this case, I have described a spectrum of cellular and acellular changes within the eyes of one patient and have established clinical and histopathologic definitions for the different types of degenerative changes seen.

"Typical" drusen are the easiest to define. The deposits are smooth surfaced and globular, and are separated from RPE by the basal lamina. They are of variable size, the larger deposits being associated with overlying RPE atrophy, accounting for the clinical findings that some are hyperfluorescent and others are not. Excessive

generalized deposit of basement membrane material, intimately related to and inseparable from the basal lamina of the RPE has been correlated by Gass with an angiographic appearance of a "stars in the sky" pattern (412A) in which hyperfluorescence occurs very early in the angiogram with many more lesions are present than are identifiable clinically as is the case in our patient. Avascular RPE detachment is invariably accompanied by excessive basement membrane production suggesting an aetiological progression as was first proposed by Sarkis (1997). RPE detachment has been described by others in association with such a deposit (79A,451) but the deposit has confusingly been referred to as drusen (604,997,1244).

The sub RPE material in the subretinal space of the pseudophakic eye must have originated from the choriocapillary circulation and from the sub-RPE neovascularisation, since in the late phase of the fluorescein angiogram, there was intense leakage of fluorescein from the circulation and red blood cells were seen in the subretinal space in association with the deposit histopathologically.

The dysfunctioning RPE did not clear the fluid from the sub-retinal space, and the globules of fluid present may represent a resolving serous detachment. An active mechanism for removing fluid from the sub-RPE space is not known, but fluid is passively reabsorbed down the osmotic pressure gradient into the choriocapillaris (955). However, I propose that thickening of Bruch's

membrane and sclerosis of the choriocapillaris with focal obliteration widened the diffusion barrier preventing the complete reabsorption of fluid.

The form of RPE decompensation shown here may be the histopathologic correlate of a clinical entity recently described (563A). There is no previous description of fluid accumulation around the RPE in a similar pattern in a human case, but McKechnie and Foulds (780A) showed a very similar appearance in a rabbit with pigment epitheliopathy induced by exposure to intense white light.

The figure (Fig. II 20B) showing sub RPE neovascularisation is very similar to one shown by Killingsworth and Sarks, but interpreted as a giant cell (609).

This case characterises two different morphological appearances of RPE detachment, and also provided an opportunity to study the spectrum of aging and degeneration changes in the choriocapillaris, Bruch's membrane and RPE.

2 Retinitis Pigmentosa

Introduction

This case demonstrates an unusually extensive deposit in Bruch's membrane in a case of dominantly inherited retinitis pigmentosa which has been published (254). My own participation in this report extended to the examination of the eyes of the first case by light microscopy, the clinical examination of the second case prior to his death, and the examination of the remainder of the family. The electron microscopy of the first case was done by Dr. McKechnie, and all the histological studies of the second case by Professor Marshall's laboratory. The details are included for completeness, with their permission.

Retinitis pigmentosa is the name given to a heterogeneous group of inherited retinal disorders in which an insidious degeneration of the outer retina gives rise to a number of common subjective symptoms and clinical signs. The literature reflects the conglomerate nature of the syndrome in terms of both diversity and conflict in account of either symptoms or pathology. Given the heterogeneity of the retinitis pigmentosa group and the range and number of functional faults that could result in the insidious loss of photoreceptor cells (79) it is perhaps surprising that in the established literature on the histological features of retinitis pigmentosa (182,265,390,720,1156,1201) significant disturbances in Bruch's membrane have not been recognized. More recently,

there have been reports of patchy deposition of material under the retinal pigment epithelium in retinitis pigmentosa (793,991) but these were not thought to differ significantly from those expected, given the age of the patients. For the first time in dominant retinitis pigmentosa, a much more widespread and uniform deposit present in the eyes of two brothers is described in this report.

Clinical Details

One patient (DH), was a male aged 62, who presented in 1973 with gradually progressive nyctalopia which had become apparent in the previous two years. His general health was good, and in particular, he was not diabetic or hypertensive. His visual acuity at that time was 6/12 N5 in the right eye and 6/18 N8 in the left eye. The media were clear. The optic disc and retinal vasculature were normal, but there was symmetrical pigmentary disturbance from the equator backwards, involving the posterior pole of both eyes, with areas of pigment clumping in a bone spicule pattern, and separated by areas of depigmentation. Visual field examination (Fig. II 35a) revealed a ring scotoma. The EOG was flat and there was a reduction in the a and b waves on the ERG in both eyes, suggesting tapetoretinal degenerative disease.

The condition was progressive, and by August 1978, his visual acuity was 6/24 in the right eye and 6/60 in the left, with small islands of visual field remaining

Fig. II35(a). Visual field chart of DH at time of presentation.

(b). Visual field chart of DH 5 years after presentation.

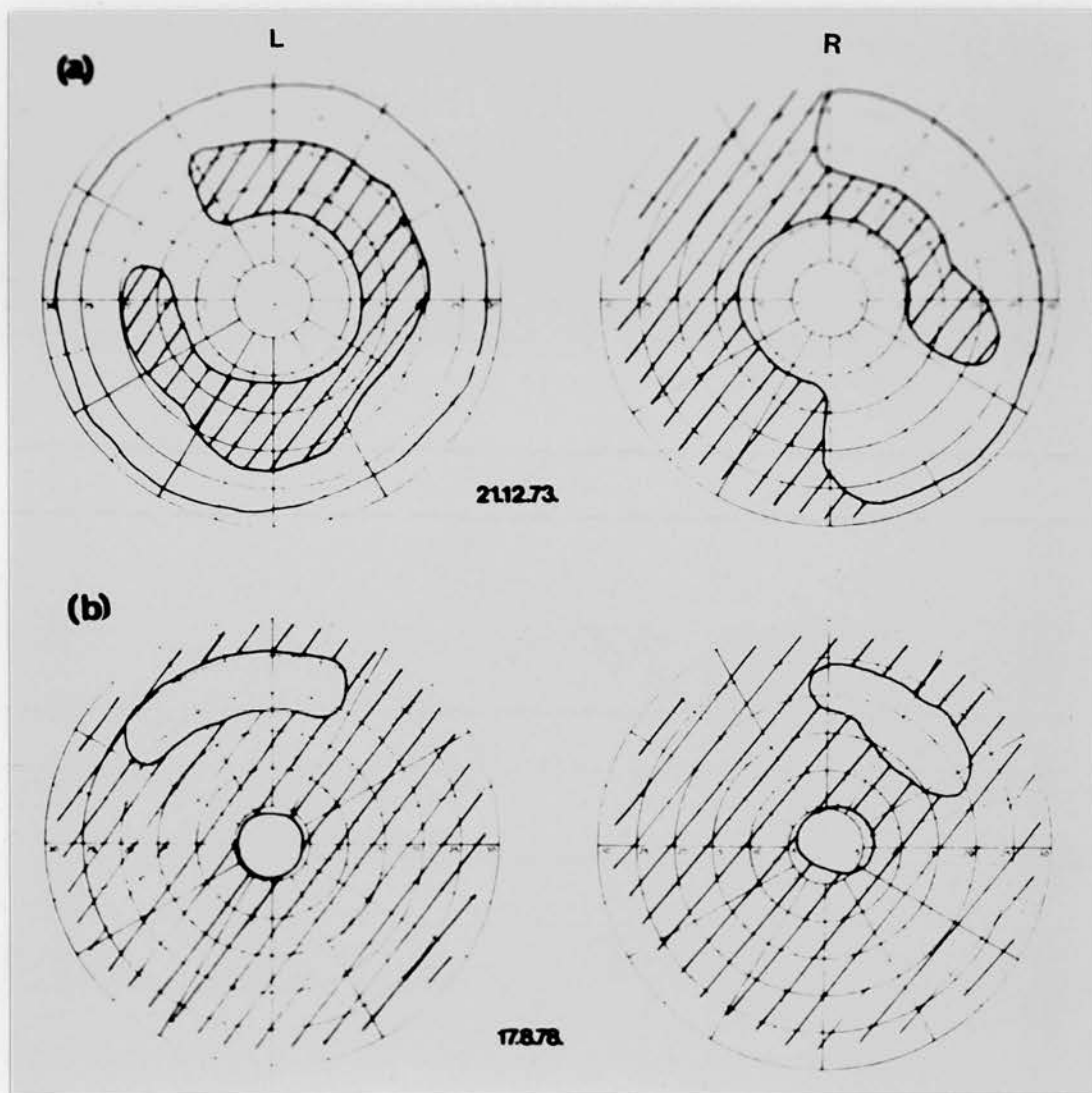


Figure II 35

centrally and in the upper temporal field (Fig. II 35b). The fundus changes were as previously recorded.

The patient died in 1981, following presumed acute myocardial infarction. There was no autopsy, but the eyes were offered for examination.

The other patient (MH) was the brother of DH and had a similar history of night blindness beginning at the age of 55 which progressed over the next twenty years to a visual acuity of counting fingers in a small superotemporal island of field in each eye. The media were clear. There was a widespread equatorial and posterior polar pigmentary disturbance (Fig. II 36) as described in his brother, but no dense bone spicule formation.

Fluorescein angiography of the fundi of MH showed two features. On both the central and peripheral aspects of the "bone spicule zone" the transitional region between presumably marked disease and mild disease was characterised by the presence of numerous sharp-edged, circular areas of pigment epithelial cell loss. Within the "bone spicule zone" there was limited masking of choroidal fluorescence, presumably where pigment granules had clumped either within the neural retina or pigment epithelium. Between these densely pigmented areas were windows of apparent hyperfluorescence where pigment was absent. There was no evidence of diminished choroidal perfusion and no abnormality of the retinal circulation.

Fig. II36A, B. Fundus photographs of MH showing focal nature of loss of the pigment epithelium and bone spindle distribution of pigment migration. The blood vessels are attenuated.

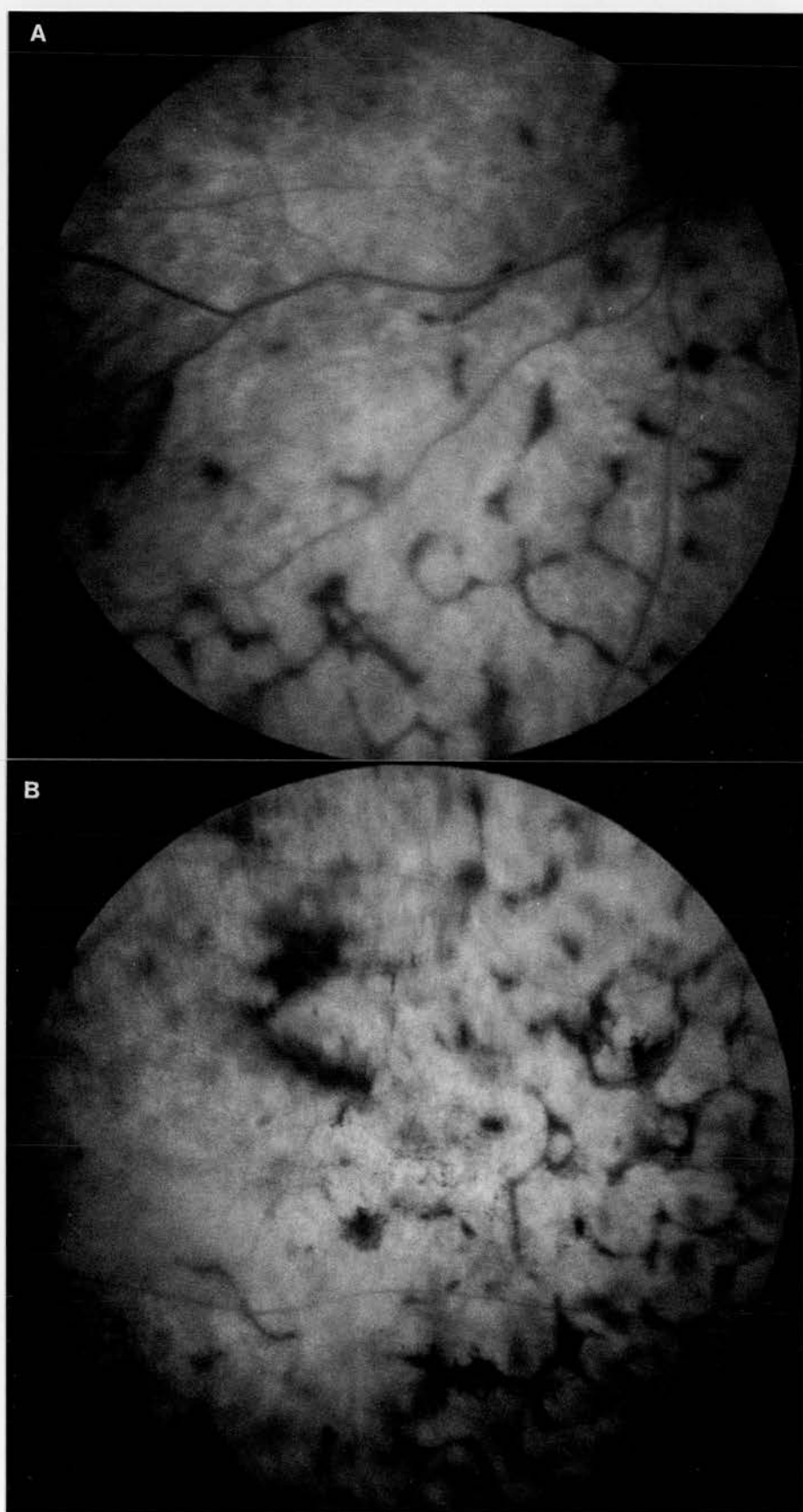


Figure II 36

This patient died in 1984 aged 74 years and his eyes also were obtained for laboratory examination.

The brothers' father had become blind in late middle-age, as had their grandfather at an undetermined age, but the diagnosis was unknown in both cases. The other living members of the family have been examined and show no evidence of disease. The family tree is shown in Fig. II 37, and this together with the clinical findings, leads us to a diagnosis of a form of dominant retinitis pigmentosa.

Materials and Methods

Ocular Histopathology

The eyes of DH were enucleated ten hours after death and were fixed immediately in 10% formal saline.

Those of MH were enucleated within four hours of death and one was processed for tissue culture while the other was fixed in 2.5% glutaraldehyde buffered in 0.1M sodium cacodylate containing 10mg/ml calcium chloride and with a final pH of 7.4.

Microscopic Examination

All four globes were of normal size (23x23x24mm).

Case 1 - DH

Both eyes of DH were opened in the horizontal plane. The anterior segments were of normal appearance and the lenses were clear. There was indistinct clumping of pigment in the postequatorial retina, including the macular region. The choroid, sclera, optic nerve, and posterior ciliary vessels were unremarkable.

Sections from the pupil optic nerve blocks were examined after staining with the following: hematoxylin and eosin, period acid-Schiff, picromallory, elastic van Gieson, phosphotungstic acid hematoxylin, Martius scarlet blue, Congo red, Bodian and Loyez. Unstained sections were examined by fluorescein microscopy using a Leitz UV photomicroscope with an HBO 200 mercury bulb. The excitation wavelength was 365nm (Leitz UGI) and a 430nm barrier filter was used.

Tissues from the calottes, including samples from the pars plicata, pars plana, ora serrata, equatorial retina, and posterior polar retina, were processed for transmission electron microscopy. The tissue, which was originally fixed in formal saline, was transferred to 2% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2) for one hour. The blocks were secondarily fixed in osmium tetroxide (1.0%) in 0.1M sodium cacodylate, dehydrated through a graded series of acetones and embedded in Transmit EM resin.

One to 2 μm sections were stained with toluidine blue and 50 to 80 nm sections stained with uranyl acetate and lead citrate were examined using a Phillips 301 electron microscope.

Case 2 MH

The glutaraldehyde fixed eye of MH was hemisected by a circumferential incision at the ora serrata.

Macrophotography was undertaken on the posterior portion of the globe and confirmed the pigment disturbances seen on ophthalmoscopic observation. The gross morphology of the globe was otherwise normal.

Tissue samples were isolated from the posterior portion of the hemisected globe by dissection using a dissecting microscope. Specimens were obtained on a horizontal axis extending from ora to ora and passing through the macula, and vertically from ora to ora passing through the optic nerve head. The location of all samples were recorded and individual pieces of tissue were cut in such a fashion that their exact orientation and relationships to other samples could still be determined even when they had been embedded in plastic. Isolated samples were postfixed for one hour in 1% osmium tetroxide buffered in 0.1M sodium cacodylate before being dehydrated in an ascending series of concentrations of ethanol and embedded in Araldite (CY212) via epoxypropane. Thick sections 0.75 - 1 μm for light microscopy were stained with Toluidine blue. Ultra thin sections were stained

Fig. II₃₇. Family tree showing autosomal dominant mode of inheritance of retinitis pigmentosa.

Fig. II_{38(a)}. Peripheral retina showing linear deposit under the retinal pigment epithelium. Note that the photoreceptors are preserved in this area, and the choriocapillaris is patent. (Picomallory x 140).

(b). Fluorescence micrograph of peripheral retina. (x 140).

(c). Equatorial retina, showing deposit with cells embedded within it, and fibrovascular material exterior to it. (Picomallory x 140).

(d). Fluorescence micrograph of equatorial retina. (x 55).

(e). Macular region showing some photoreceptor nuclei preserved but with stunted outer segments. The retinal pigment epithelium cells are more closely packed than elsewhere. (Picromally x 55).

(f). Fluorescence micrograph of macular region. (x 140).

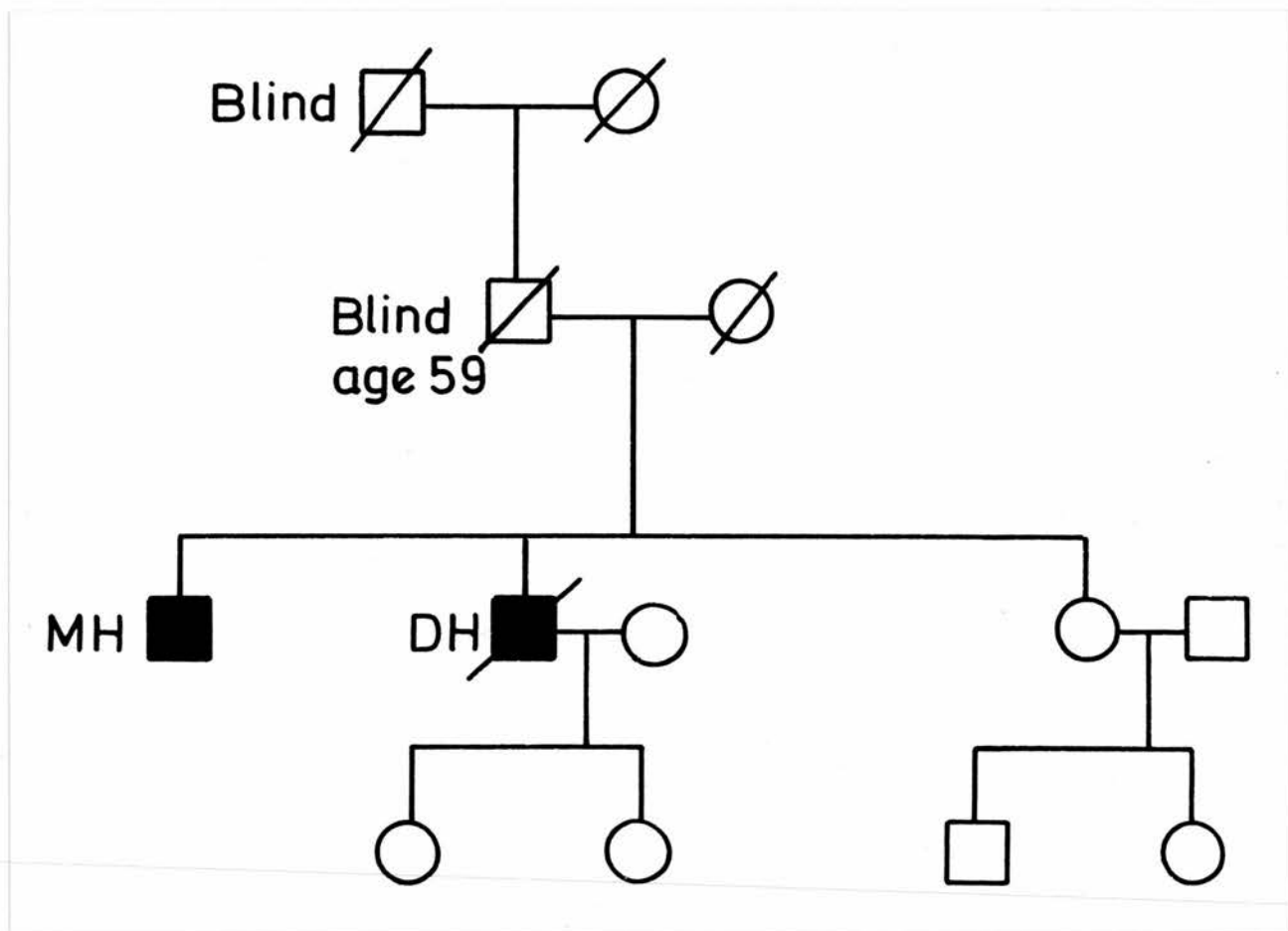


Figure II 37

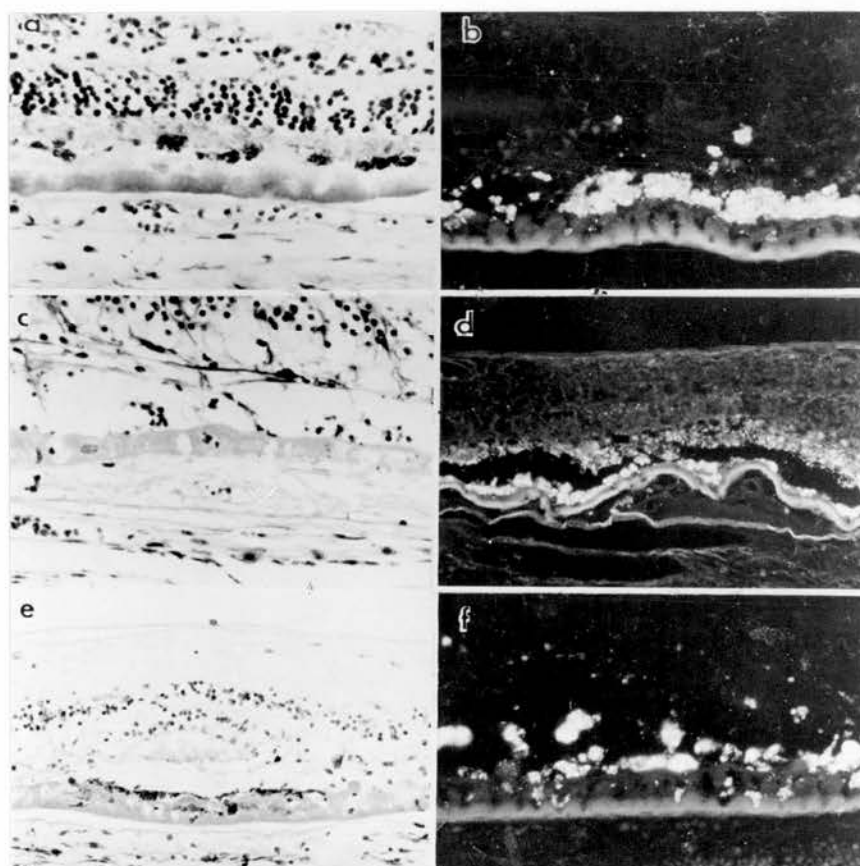


Figure II 38

with uranyl acetate and lead citrate before being examined in an AEI 801 electron microscope.

The intervening segments of posterior globe were processed for examination by scanning electron microscopy. Of these, three were postfixed in osmium tetroxide as previously described, but one was not. All four samples were critical point dried (Samdri 780) after dehydration using an acetone series. The three osmium postfixed samples were sputter coated (Emscope SC500) with 40nm of gold while the remaining one was coated with carbon. The three gold coated samples were mounted on aluminum studs and examined in either a Hitachi 510 or 520 scanning electron microscope. The carbon coated specimen was mounted on a carbon stud and examined in a Hitachi 530 scanning electron microscope fitted with a Kevex X-ray microanalysis system.

Results

Case 1 DH

Light Microscopy

The findings in each eye were essentially the same. The anterior segments were unremarkable. The basement membrane of the ciliary processes was prominent and probably thicker than normal. The basement membrane of the pars plana was thickened and strongly PAS positive.

The inner retinal layers were unremarkable. At the extreme periphery of the retina, the outer nuclear layer was preserved and the photoreceptors were atrophic (Fig. II 38a). The remainder of the retina showed a total atrophy of the photoreceptor layer except at the macula where occasional photoreceptors with stunted outer segments were seen (Fig. II 38a-f).

A thick basal deposit (Fig. II 38a-f) was present on the retinal surface of Bruch's membrane throughout both eyes. In some locations, the inner and outer margins of the deposit were seen to be bounded by the basal membrane of the retinal pigment epithelium and the elastic layer of Bruch's membrane respectively. However, in some places, the former was absent and the latter difficult to identify. The material was weakly PAS positive, and with all the stains used, was denser in the outer part than in the inner part. It did not show dichroism with Congo red and was not amyloid. The deposit was present from the disc to the ora serrata and was continuous with the deposit in the pars plana. Peripherally, it was acellular, but more posteriorly, contained a scattering of small mononuclear cells; a few large pigmented cells were embedded in it and were also lying on its inner surface (Fig. II 38c,e). The pigment within these cells exhibited the autorfluorescence of lipofuscin (Fig. II 38b,d,f). There was also some extracellular lipofuscin in the deposit and in the retina.

The inner surface of the deposit was irregular with nodules protruding towards the retina, and deep radial clefts.

In some sectors, most notably in the mid-periphery, the deposit was separated from Bruch's membrane by fibrovascular tissue (Fig. II 38c,d, Fig II 39).

There was minimal migration of pigment epithelial cells into the retina. The architecture of the inner retina and optic nerve was preserved.

The choriocapillaris was patent in most areas, but was replaced by hyaline tissue in some parts and elsewhere, there was mild hyalinization of the choroidal vessels. The choroid was atrophic in the macular region.

Transmission Electron Microscopy

The deposit, when uncomplicated by fibrovascular ingrowth, was found lying between the pigment epithelium and Bruch's membrane. Representative areas of the deposit in the mid-periphery, pars plana, and the ciliary body are shown in Figs. II 40a,b, and c respectively. The more complicated situation where there was fibrous tissue between the deposit and Bruch's membrane is shown in Fig. II 40d. Anteriorly, the deposit was composed of an almost amorphous material with small inclusions of a more electron dense material (Fig. II 40b, c). Towards the posterior pole, the granular material became more prominent (Fig. II 40a,d) and took on a frond-like

Fig. 1139 (a,b). Light micrographs showing spindle shaped cells (arrowhead) under the deposit. (Haematoxylin and eosin $\times 300$).

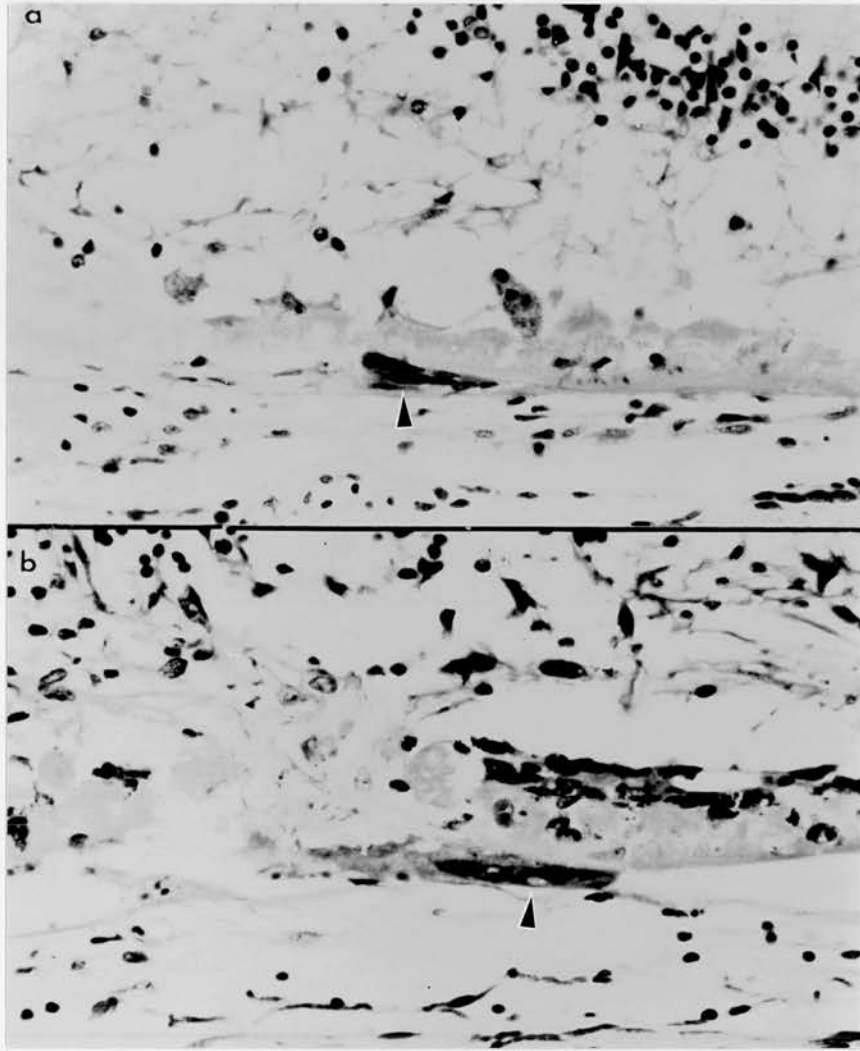


Figure II 39

Fig. II40. Ultrastructural features of the basal deposit.

A. Mid-periphery showing deposit between retinal pigment epithelium and Bruch's membrane. The major part is formed of fibrous fronds with amorphous material between (arrow). BM, Bruch's membrane. CC, choriocapillaris. N, Pigment epithelial cell nucleus. (x 1650).

B. Pars plana showing deposit (arrow). NPE, Non-pigmented epithelium; PE, pigmented epithelium. (x 1000).

C. Pars plicata showing similar deposit, here mainly amorphous but with a fibrous component (arrow) E, endothelial cell; PE, pigmented epithelium. (x 2650).

D. Posterior pole. The deposit here shows some large spaces (arrow) occupied by large cells in some sections. There is fibrovascular tissue lying between the deposit and Bruch's membrane (BM). Note that the Choriocapillaris is patent. PE, pigment epithelium; D, deposit; CC, choriocapillaris; BV, blood vessel. (x 980).

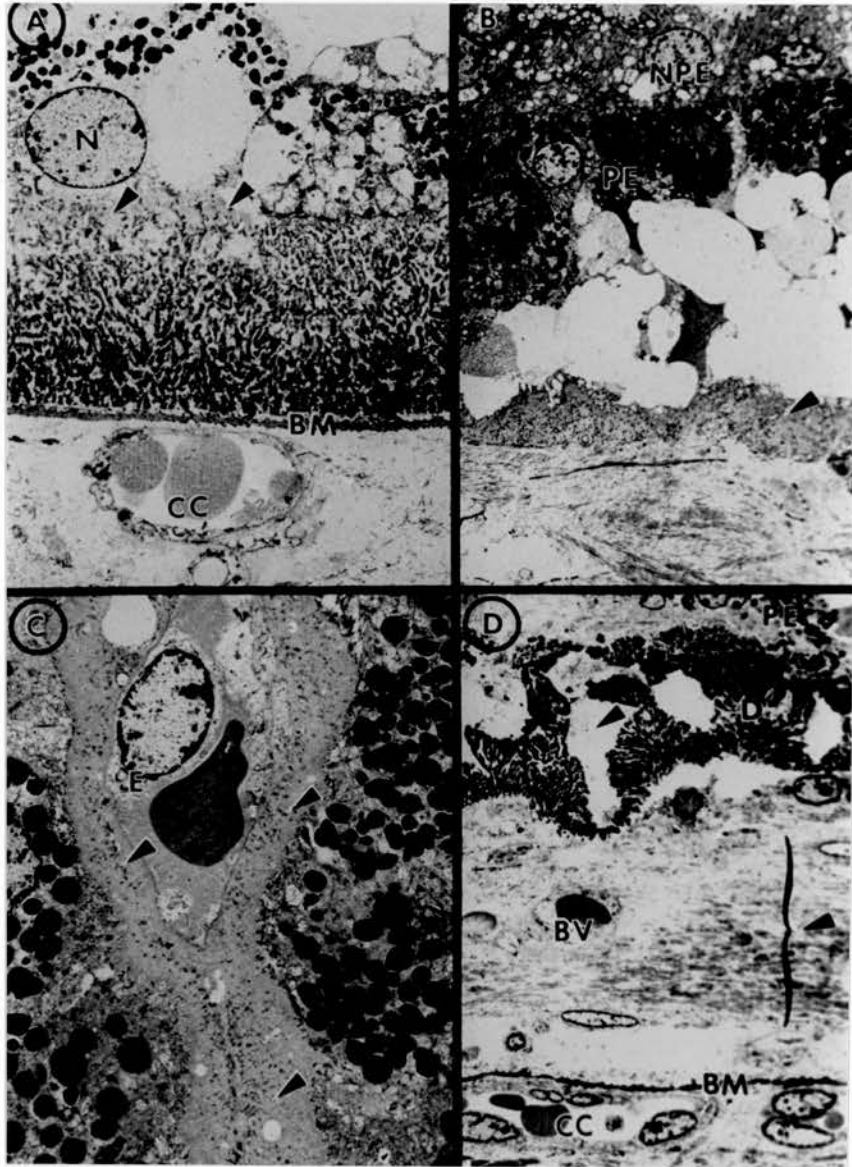


Figure II 40

appearance. This frond-like material was often split by radial clefts. Degenerated cells were seen in these clefts, but identification of cell type was impossible due to the advanced autolytic changes. The fibrovascular tissue seen under the deposit (Fig. II 40d) contained identifiable fibroblasts and capillaries and some cells which may have been macrophages. In the areas examined, the choriocapillaris was present and appeared to be patent.

At high magnification, the outer layers of Bruch's membrane were of unremarkable appearance (Fig. II 41a), however, the basement membrane of the pigment epithelium, which normally forms the innermost layer of Bruch's membrane, was absent (Fig. II 41b). The frond-like material, which formed the bulk of the deposit posteriorly, was particulate in structure when examined at very high magnification (Fig. II 41c). The material which was found external to the retinal pigment epithelium and which appeared amorphous at low magnification (and formed the bulk of the deposit anteriorly) had a filamentous structure when seen at very high magnification (Fig. II 41d). The filaments had a diameter of 10 to 13 nm.

Case 2 MH

Light Microscopy

The range of histopathological findings observed in the eyes of DH were mirrored almost exactly in the one eye of his brother that was similarly examined. The most

Fig. II41. Electron micrographs illustrating the fine structures of the basal deposit shown at lower magnification in Fig. II40.

A. Bruch's membrane showing some ultrastructural features. (x 28,000).

B. The outer aspect of the deposit. Note that the pigment epithelial basement membrane appears to be absent. (x 51,000).

C. The frond-like material appears to be particulate in nature at high magnification. (x 35,000).

D. The amorphous material seen at lower magnification, between the frond-like material appears filamentous at high magnification. The filaments are about 10-13 nm in diameter. EBM, endothelial basement membrane; OC, outer collagenous layer; elastic layer; IC, inner collagenous layer; D, deposit. (x 98,000).

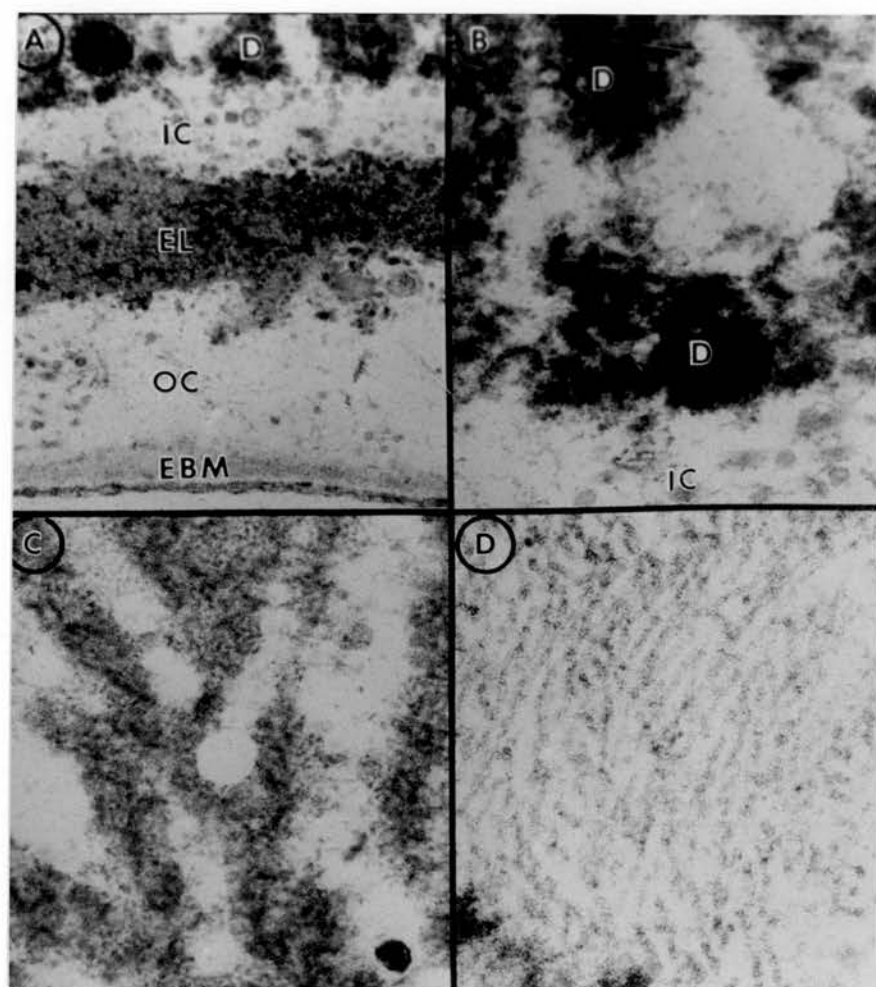


Figure II 41

notable exception was that in MH, there were relatively larger areas of photoreceptor cells preserved throughout the peripheral retina (Fig. II 42). Both rod and cone cells were present, but both exhibited atypically short outer segments with disorganised disc membranes (Fig. II 43). Towards the central margins of this peripheral zone of photoreceptor, their outer segments became progressively shorter and increasingly deranged, especially at their outermost ends. Even within the paracentral zone where photoreceptor cells were for the most part lost, small cave-like regions were occasionally observed and seen to contain remnants of both inner and outer segments (Fig. II 43). In these regions, the roofs of caves were delineated by the outer limiting membrane which curved to be in opposition to the pigment epithelium around the margin of each cave (Fig. II 43). These degenerate vestiges were identical to those observed in the macular region of DH (Fig. II 38e). Throughout the retina, photoreceptor cells were never found in areas where pigment epithelial cells were absent.

The inner layers of the neural retina were for the most part well-preserved and showed few abnormalities that could not be related to the patients' age or the postmortem time of this sample. The major atypical feature was the presence of numerous cells on the vitreal aspect of the inner limiting membrane. Unlike most other reported cases of autosomal dominant retinitis pigmentosa, in MH, there was little condensation of pigmented cells within the neural retina, and only

Fig. II42. Light micrographs of (a) peripheral and (b) para central areas of retina of MH. The inner retinal layers were relatively well preserved in all samples and in all locations the subpigment epithelial deposit was present (arrowed). (x 480).

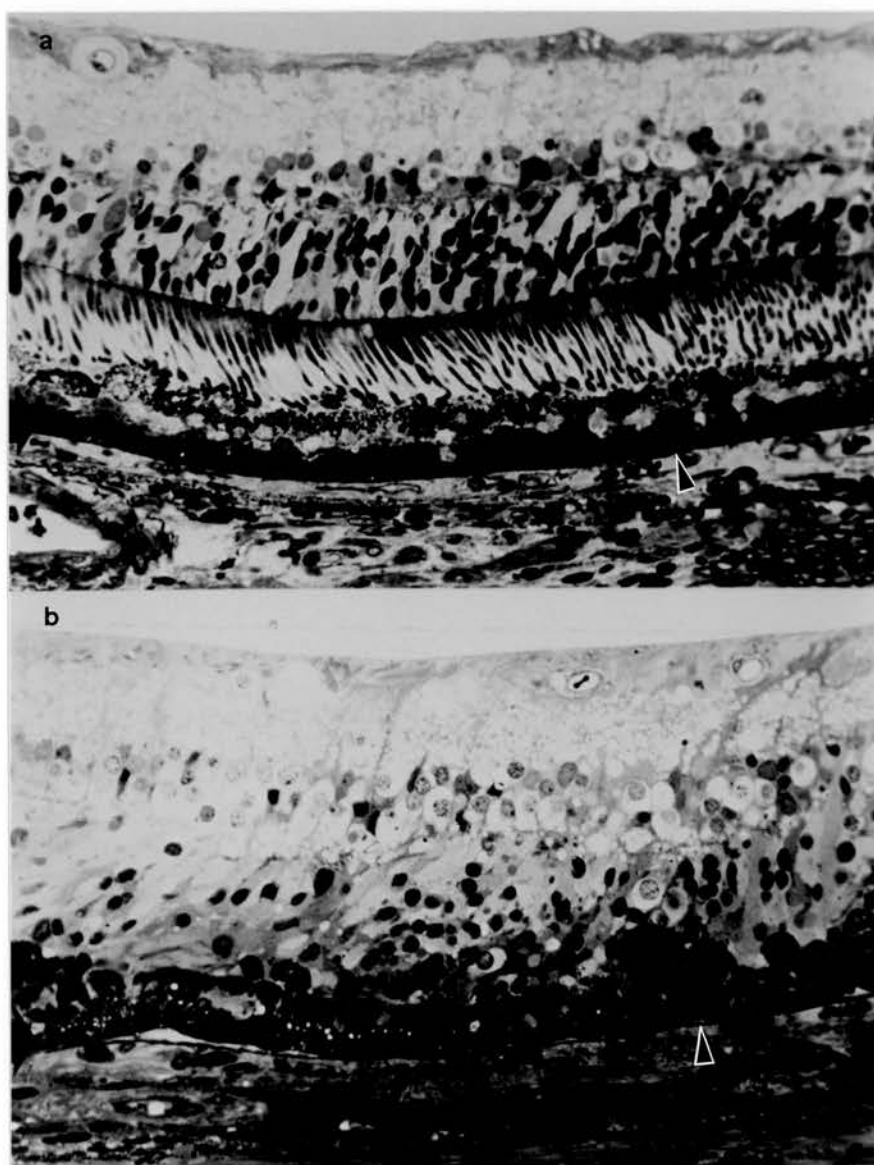


Figure II 42

Fig. II43. Light micrographs (a, b, c); and scanning (d) and transmission (e) electron micrographs of the interface between photoreceptor cells and retinal pigment epithelium in the paracentral retina of MH. The vestigial photoreceptor cells always showed disorganised outer segments. At the edges of the paracentral zone were many small "caves" of photoreceptor cells overlying islands of pigment epithelial cells (c). The subpigment epithelial deposit (arrowed) varied in thickness but was always present. (a, b, c \times 1,000; d \times 12,000; e \times 6,000).

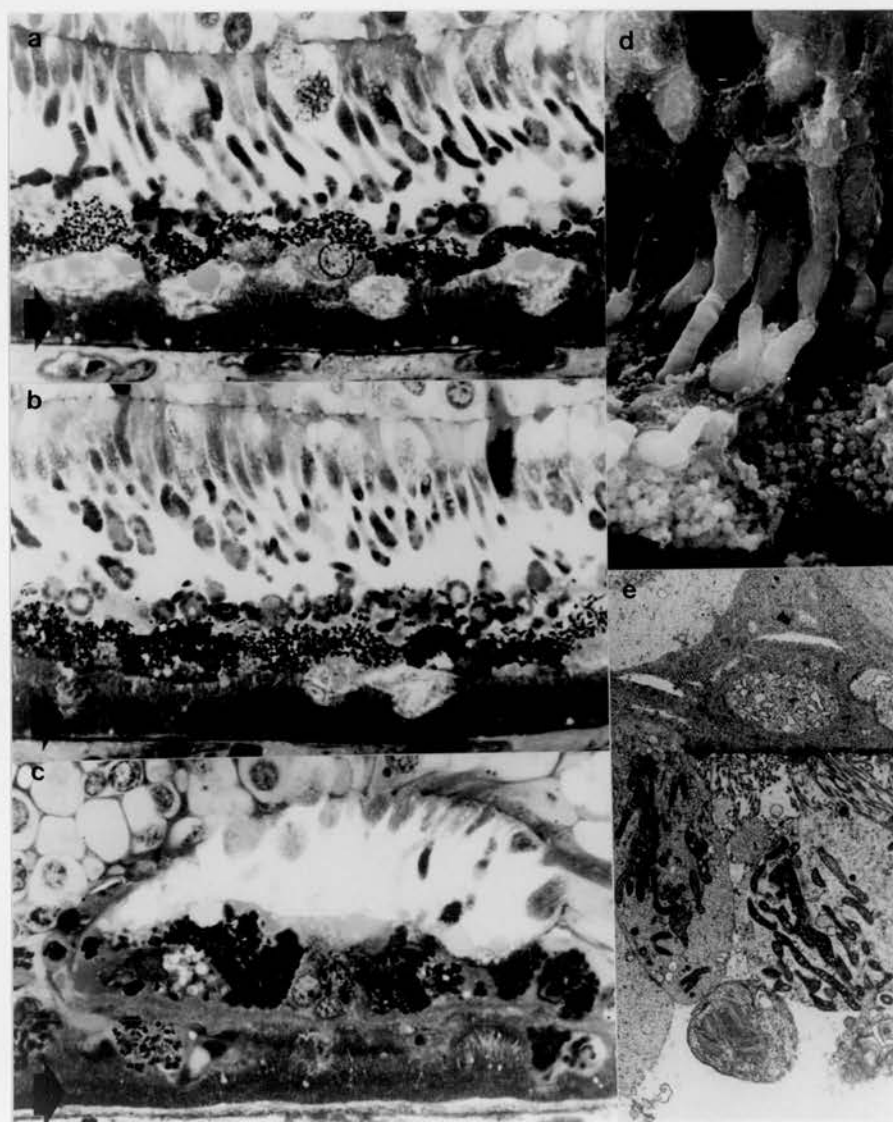


Figure II 43

occasional rounded cells containing pigment in the subretinal space.

The retinal pigment epithelium showed a discontinuous distribution, and was absent over large areas of the bone spicule zone (Fig. II 42). In contrast, the subpigment epithelial deposit was ubiquitous and varied only in its thickness (Fig. II 42, 43, 44). Transition between areas of pigment epithelial presence and absence were always abrupt and especially so in relation to the numerous receptor caves (Fig. II 43c). In these foci, islands of pigment epithelial cells lined the caves and were associated with morphologically recognisable remnant of photoreceptor cells. In the surrounding tissue immediately adjacent to such locations both pigment epithelium and photoreceptor cells were absent and inner retinal elements were directly juxtapositioned to the subepithelial deposit. Throughout this globe, all remaining pigment epithelial cells were compressed in their apicobasal aspect, and both melanin and lipofuscin granules were more rigidly confined to the apical aspect of the cells than in the case in normal age-matched controls.

The subepithelial deposits in MH were identical to those in DH in terms of gross morphology, location, and distribution. The staining patterns were also similar with an outer band staining intensely and pale staining inner band. In some regions in DH, particularly in areas of pigment epithelial cell loss, this bilayered staining pattern was duplicated with apparently two subepithelial

Fig. II44. Light micrograph (a) and scanning (b) and transmission (c, d) electron micrographs of the subpigment epithelial deposit in MH. In some areas the dichromatic staining pattern of the deposit was duplicated (open arrows). Numerous mononuclear cells were seen within the deposit (large closed arrows). Electron micrographs show the presence of small globular inclusions (small closed arrows) within the granular fronds of deposit (a x 2,500; b x 5,000; c x 17,000; d x 25,000).

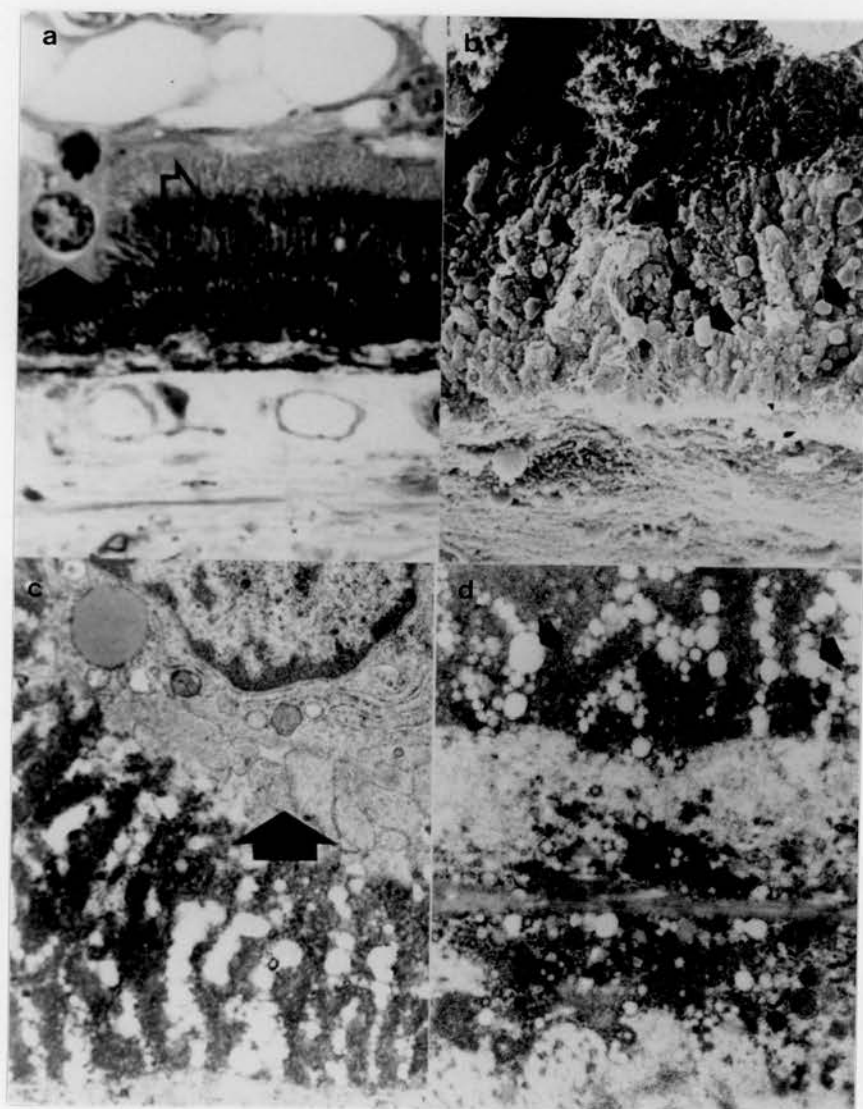


Figure II 44

deposits being superimposed one upon another (Fig. II 44a). As in DH, occasional mononuclear cells were found in this region, but in contrast to DH, no evidence was seen of multinucleate cells.

Electron Microscopy

Both scanning (Fig. II 43d) and transmission electron microscopy (Fig. II 43e) of vestigial photoreceptor cells showed a significant shortening and disorganisation of the outer segments. In many instances, the cross-sectional areas of the outer segments of both types of photoreceptor cell was increased in size due to dislocation and convolution of the contained discs. This was especially noticeable at the outermost tip of the outer segments of rods (Fig. II 43d).

The frond-like appearance of the subretinal deposit was particularly apparent in scanning electron micrographs and its structure was reminiscent of coral (Fig. II 44b). A topographic analysis of the surface seen in such specimens suggested that the deposit had at least two components, the major component giving rise to the granular surfaced columns of the fronds, and a minor constituent which was responsible for the smooth surfaced spheres randomly distributed over the fronds (Fig. II 44b). This bimodal structure was also seen in the transmission electron micrographs where the major components of the fronds of subepithelial deposit were granular and electron dense, and between fronds, globular pale areas were observed (Fig. II 44c,d). The outermost

aspect of the deposit was adjacent to the inner collagenous layer of Bruch's membrane, and as in the first case (DH), the basement membrane of the pigment epithelium was either displaced or lost (Fig. II 44d). The apical surface of the deposit varied in relation to presence or absence of pigment epithelial cells. In their absence, the surface was usually in contact with retinal glial elements or mononuclear cells (Fig. II 44c), and usually relatively less convoluted. Where epithelial cells were present, the convoluted basal border of these cells sent deep processes into the clefts between the fronds of deposit. The undulating and highly convoluted surface induced by such ingressions could best be reviewed in scanning electron microscopy specimens in which the overlying pigment epithelial cells had been either naturally or artificially removed (Fig. II 45). The frond-like appearance has been reported in two cases of basal linear deposit (714A, 1216).

The limited analytical electron microscopic microanalysis carried out on specimens of subpigment epithelial deposits were disappointing in that no clear results were obtained in relation to the elemental composition of the deposits. The X-ray emissions from the specimen suggested slightly elevated levels of both iron and calcium, but these indications were not highly significant.

Discussion

Fig. 1145. Scanning electron micrographs of the surface of pigment epithelium and subpigment epithelial deposits in the retina of MH.

A. Shows engorged epithelial cells, most with relatively normal hexagonal appearance and lateral dimensions, but some with large holes in their apical surface. Note the broken receptor tips (arrowed) adherent to these cells. (x 2400).

B. Shows retinal pigment epithelial cells (A), and the exposed surfaces of the subretinal debris (B) and Bruch's membrane (C) respectively. The imprinting of the artificially displaced epithelial cells on the debris can be clearly seen. (x 1250).

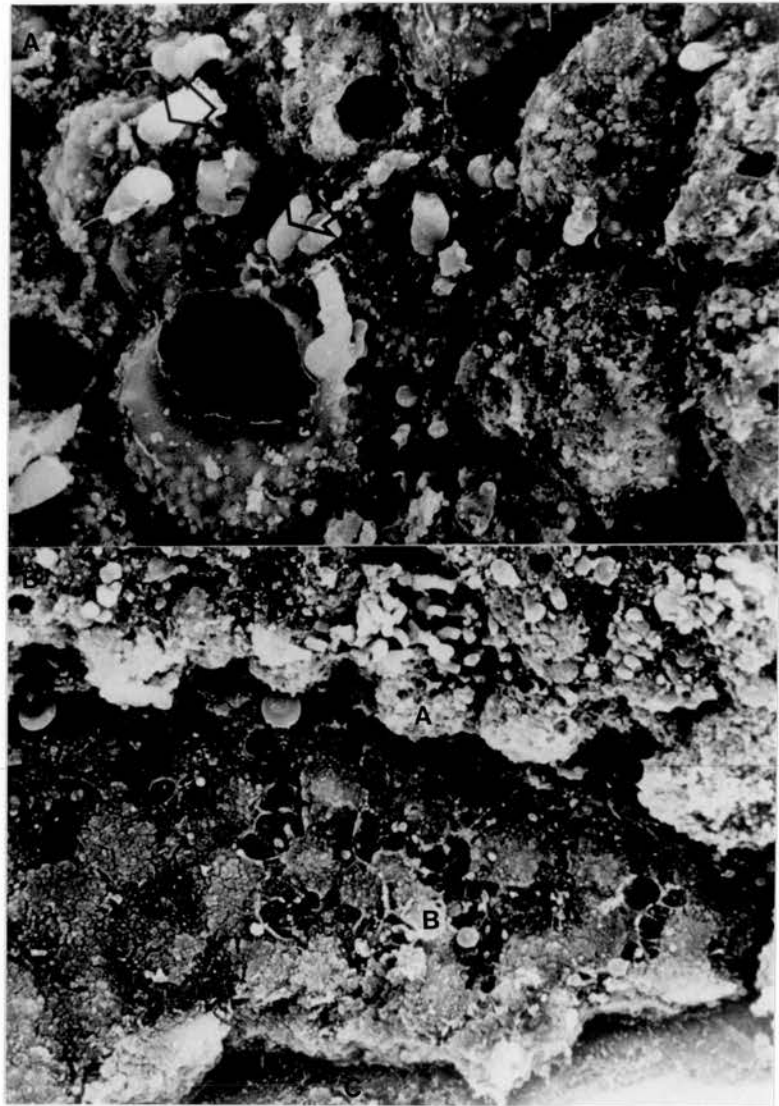


Figure II 45

The clinical history and physical findings in both brothers in the present study are consistent with those of dominantly inherited retinitis pigmentosa (323), even though the age of onset is older than usual, the retinal vessels and optic disc were normal, and the bone spicule pigmentary disturbance involved the macular area as well as the equatorial retina. Such deviations in relation to particular characteristics are not unexpected as it is well known that these diseases may have features that vary from one family to another, and even between individuals within the same family.

The striking difference between these two cases and previously reported histological findings in autosomal dominant retinitis pigmentosa, is widespread and uniformly thick subretinal pigment epithelial deposit.

These two cases show some features typical of classical retinitis pigmentosa, namely the midperipheral distribution of photoreceptor cell loss with a relative sparing of the far periphery and the macula; pigment migration into the retina (although in the cases presented here it was not a prominent feature) and preservation of the choriocapillaris (182,265,390,720,1156,1201). In contrast, the classical descriptions of the histological appearances of cases of retinitis pigmentosa (182,265,390,720,1156,1201) describe Bruch's membrane as being of normal appearance when related to the age of the subject (390), and there is no mention of any subpigment epithelial deposits in most of the ultrastructural studies of these diseases (631,817,1095).

Similar deposits to that currently described have been identified in three previously published cases of retinitis pigmentosa, but in none of these reports were the deposits as extensively distributed as in our cases, and associated subretinal neovascularization has not been reported. Of these previous cases, two were of dominantly-inherited disease (793) and one was X-linked (991). In all three cases, there was a focal loss of photoreceptor cells in relation to areas where the deposit was not covered by pigment epithelial cells, and in such foci, no abnormalities were observed in the underlying choriocapillaris. Santos-Anderson and colleagues, (991) described the deposit as atypical and thickened basement membrane.

It is unlikely that in the cases we present, the greater extent of the abnormal deposit simply represents a more advanced stage of the disease than the other three reported cases, since all three of those cases had symptomatic histories of thirty to forty-five years, compared to ten and twenty years in our cases, and all had worse visual acuities than our cases when last tested prior to death.

Deposition of amorphous material under the retinal pigment epithelium and in the inner collagenous layer of Bruch's membrane has been described in Doyme's choroiditis (196,1205) and leukemic infiltration of the choroid (1205) and has been presumed to be secondary to disease in the choroid. Ashton and Sorsby (42) agreed

with this view in describing a deposit similar to that in our cases, but again, less extensive, in a case they term fundus dystrophy.

Deposition of abnormal material at the level of Bruch's membrane with thickening and increasing PAS positive staining are well recognized aging changes (340,371,521,622,994). The deposit in the cases presented differed from that described by Sarks (994) in that in our patients, first it was much thicker and more widespread (Sarks, personal communication) and secondly, it was located internal to the inner collagenous layer of Bruch's membrane, rather than within it. The geographic distribution of the deposits in our cases was also quite unlike that observed by Sarks (994) in that it was fairly uniformly distributed from the ora to the optic nerve, rather than being centered at the posterior pole as in senile eyes. We agree with the suggestion of Santos-Anderson and her group, (991) that this was a basement membrane deposit, since it replaced the basement membrane of the retinal pigment epithelium rather than lying on one or other side of it. Deposit with this unusual pattern of fronds and clefts has been described in age related macular degeneration (714A) and in association with familial drusen (249).

Subretinal neovascularization, which we found in the outer aspects of the deposits in some areas of DH, is not a recognized feature of retinitis pigmentosa, but is seen in association with basal laminar deposit in 20% of eyes of the elderly (993).

At one point in DH, we observed a break in the deposit, with glial proliferation thorough the break, possibly representing an early stage of neovascularization. the stimulus for neovascularization is unknown, but it could be due to a variety of factors, such as; an inflammatory response to the deposit or to an unidentified chemical mediator of neovascularization present in the deposit; or as a response to ischemia induced in the retinal pigment epithelium as a result of the increased diffusion barrier for oxygen created by the deposit between the choriocapillaris and the retinal pigment epithelium. Absence of the RPE may have promoted neovascularisation once started since it normally inhibits endothelial proliferation (149).

I suggest that the deposit in *this* cases was a result of a disturbance of retinal pigment epithelial metabolism rather than material derived from the choroid. If it were derived from the choroid, it should perhaps show patchy distribution which related to choroidal vascular patterns, and as a consequence, perhaps should be most concentrated at the posterior pole. In the cases of Meyer et al (793) the deposit was present only in those areas where the retinal pigment epithelium was present. The presence of lipofuscin within cells in the deposit lends additional support to the view that the defect was primarily at the level of the retinal pigment epithelium. The retinal pigment epithelial cells were presumably incorporated in the deposit as it was laid down. The presence of a similar deposit under the ciliary

epithelium and pars plana was suggestive of disturbed metabolism in a variety of cells with a common ancestry. The nature of the metabolic fault is unknown, but the deposit could arise through any one of a number of enzymic defects in systems concerned with lysis of phagocytic inclusions; or from problems in the voiding mechanisms implicit in the normal phagocytic load of the retinal pigment epithelium (79).

Retinitis pigmentosa is a term encompassing many different conditions having as a common feature a disturbance of pigmentation of the fundus (79). In the knowledge of the diversity of clinical manifestations of the disease, it is not surprising to find that the histological appearances differ. We present these cases of dominant retinitis pigmentosa with the unique findings of a widespread subretinal pigment epithelial deposit. There were no clinical features to differentiate this form of disease from retinitis pigmentosa with the classical histological findings.

3. Glomerulonephritis

In this case, a direct relationship between ocular and glomerular pathology is described. The patient had type II mesangiocapillary glomerulonephritis. This case has been submitted for publication in collaboration with Dr. McKechnie, who performed the electron microscopic studies on the eye and Dr. MacDonald who studied the kidney pathology.

Glomerulonephritis can be classified according to the histopathological appearances. In type II mesangiocapillary (MCGN Type II) glomerulonephritis, a deposit, characteristically electron dense on electron microscopy, is seen in the glomerular capillary basement membrane. I report here the clinical case and histopathological findings in the eyes of a patient with MCGN type II, in which a deposit of abnormal material in the choroid and Bruch's membrane similar to that demonstrated in the glomerulus, was present. The choroid and the kidney have anatomical similarities to each other in that they both have a large capillary bed made up of fenestrated vessels, and I suggest that the two sites may be affected by similar disease processes.

Clinical history

An 18-year old male patient presented in March, 1981, with a two week history of malaise, leg cramps, ankle swelling and nocturia. On renal biopsy, his nephrotic syndrome was found to be due to Type II mesangiocapillary

glomerulonephritis. He was initially treated with diuretics, a low-salt diet, and hydralazine to control his blood pressure, which was raised to 150/110 mm Hg at presentation. His renal function, however, continued to deteriorate and in July, he was started on plasmaphoresis and immunosuppressive treatment. Having been normotensive since his initial presentation, he became hypertensive in September, with a blood pressure of 180/120 mm Hg supine, 120/100 mm Hg erect. The blood pressure was controlled within a few days with prazosin and metoprolol. However, during that time, he complained of blurred vision, and was found to have corrected visual acuities of 6/24 in each eye. The anterior segments were normal. He had bilateral papilloedema, macular oedema, and scattered flame-shaped haemorrhages. Over the next month, the vision in the right eye deteriorated to hand movements only, while in the left, it improved to 6/12 with gradual resolution of the signs. At about this time, he was started on haemodialysis.

On 9th July, 1982, he returned to the eye department with a complaint of pain in the right eye which occurred only while on dialysis. There was no perception of light in the right eye, an afferent pupil defect, corneal oedema and an intraocular pressure of 52mm Hg. The left eye had a visual acuity of 6/9, with no fundus abnormalities. The patient was treated with topical steroid, atropine 1%, and timolol 0.5%, but he continued to complain of severe intermittent pain while on dialysis. On 27th July, when he was seen again, he was found to have a massive exudative bullous retinal detachment. The

intraocular pressure remained elevated and he was admitted to hospital for attempted medical control with the addition of acetazolamide. This failed, as did two retrobulbar injections of alcohol, to alleviate his pain, and the right eye was enucleated on 10th August, 1982. Fluorescein angiograms of the left eye on 6th September showed no disturbance of choroidal or retinal circulations. There were, however, numerous hyperfluorescent discrete foci at the posterior pole and concentrated in the perifoveal area (Fig. II 46), fluorescing early in the angiogram, and becoming more intensely fluorescent throughout. These are the angiographic appearance of drusen.

The patient had remained well on continuing renal dialysis with no change in the appearance of the left eye, but he collapsed and died on 9th April 1983. Autopsy revealed no other cause of death and it was concluded that he had had a cardiac arrest or arrhythmia. Examination of the kidneys yield no additional information. The left eye was not obtained for examination.

Renal Biopsy Pathology

Light Microscopy

There were eleven glomeruli in the biopsy. All the tufts showed the characteristic appearances of mesangiocapillary glomerulonephritis with marked mesangial increase, involving particularly the matrix,

Fig. II46. Fluorescein angiogram of left eye showing multiple hyperfluorescent foci with the characteristics of drusen.

Fig. II47. Electron micrograph of renal glomerulus. The basement membrane of the walls of these glomerular capillaries can be seen (arrow); the membrane varies in width and is abnormally electron dense. ($\times 2,000$).



Figure II 46

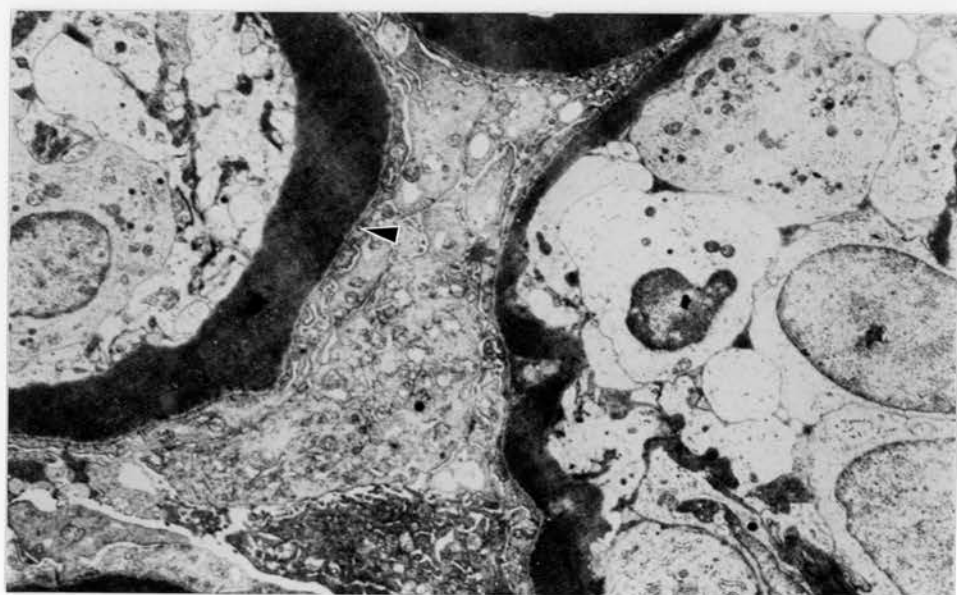


Figure II 47

but also some cellular proliferation. Long stretches of the capillary basement membrane were highly PAS-positive and thickened. Five of the glomeruli showed large epithelial crescents.

Immunofluorescence microscopy showed large amounts of C_3 and IgM in capillary walls, the latter in focal and segmental distribution. Little positive fluorescence was seen in the mesangium. C_3 and C_4 were present also in some tubular basement membranes.

Transmission electron microscopy showed the presence of an electron dense deposit throughout the glomerular capillary basement membrane, thickening the membrane irregularly in some places (Fig. II 47).

The appearances were those characteristic of type II mesangiocapillary glomerulonephritis (dense deposit disease).

Eye Pathology

Macroscopic examination and preparation of tissue

The right globe was immediately fixed in 10% formal saline. It measured 22x22x22mm with 2mm of optic nerve attached. The eye was opened horizontally, revealing a total retinal detachment with a subretinal coagulum.

The pupil optic nerve block was processed through paraffin, and sections were examined after staining with

the following: haematoxylin and eosin, periodic acid-Schiff, Bodian, Loyez, and Martius scarlet blue.

Immunoperoxidase staining for IgG, IgM, kappa chain, lambda chain, C₃ and C₄ was also undertaken.

Tissue from the calottes was prepared for transmission electron microscopy. tissue blocks of approximately 2-3mm were dissected from the following areas: pars plicata, pars plana, ora serrata, equatorial retina and posterior polar retina. In all cases, the sclera was dissected off. The tissue, which was originally fixed in 10% formal saline, was transferred to 2% glutaraldehyde in 0.1M cacodylate buffer (pH7.2) for one hour. The blocks were secondarily fixed in osmium tetroxide (1.0%) in 0.1M sodium cacodylate, dehydrated through a graded series of acetones, and embedded in transmit EM resin. Propylene oxide was used as the intermediate solvent.

Sections of one to two μm were cut and stained with toluidine blue for orientation by light microscopy. Sections from selected blocks were cut at 50 to 80 nm, stained with uranyl acetate and lead citrate, and examined and photographed with a Phillips 301 electron microscopy.

Light microscopic examination

The corneal epithelium was thinned, with intraepithelial oedema and some bullous separation. The stroma appeared normal. The endothelial cells were flattened.

The drainage angle was closed by an extensive neovascular membrane over the iris surface, with considerable ectropion uvea. There was a mild mixed inflammatory cell infiltrate in the iris and the anterior chamber was filled with homogeneous eosinophilic material. The trabecular meshwork was compressed.

There was a posterior subcapsular cataract.

The ciliary processes were atrophic with PAS-positive deposit in the basement membrane and the stroma of the processes.

The choroid was thinned, but the vessels in all layers were patent. The arterioles did not show fibrinoid necrosis (Fig. II 48). There was an extensive deposit of PAS-positive material both in the walls of the choroidal blood vessels and in Bruch's membrane, which showed both linear thickening and focal deposition, resembling drusen (Fig. II 49); this was present throughout but most marked at the posterior pole. The deposit stained positively for fibrin with MSB, unlike typical drusen. No immunoglobulin or complement could be demonstrated in the deposits.

Fig. II48. Choroid and Bruch's membrane showing both focal (f) and linear (l) thickening.

Note that the vessels in all layers of the choroid are patent, but show some deposition of material in their walls (arrow) (stain PAS, x 1,000).

Fig. II49. Focal thickening of Bruch's membrane resembling drusen formation. (Stain PAS, x 1,000).

Fig. II50. Electron micrograph of ciliary processes showing dense deposit (d) in the basement membrane. (x 1,500) (Inset x 6,000).



Figure II 48



Figure II 49



Figure II 50

The retinal pigment epithelium showed some proliferation with the formation of a cellular membrane inside the pigment epithelium. There was a total retinal detachment with extensive recent and organising intraretinal haemorrhage. the retinal architecture was completely replaced by glial cells and the subretinal space was filled with eosinophilic material showing cholesterol clefts and containing many pigment laden macrophages. Some of the retinal vessels, particularly at the periphery, showed thickening of their walls. There was an extensive fibrovascular preretinal membrane.

The optic nerve was markedly atrophic with very few nerve axons, and showed anterior demyelination. There was infarction of the nerve head, but the central vessels were patent in the levels examined. There was buried drusen in the optic nerve head.

The posterior ciliary vessels were patent, but fibrin was focally deposited in their walls.

Electron microscopy

It was confirmed that a deposit was present in the basement membrane of the ciliary epithelium (Fig. II 50) and throughout the extent of the inner collagenous layer of Bruch's membrane, where it took two forms, being both linear and focal (Fig. II 51). The deposit was present also in capillary basement membrane (Fig. II 51). The material was electron dense and amorphous, as in the kidney, although less densely staining.

Fig. 1151. Electron micrographs of Bruch's membrane showing dense deposit in drusen like distribution and also in the choriocapillaris (arrows) (x 2,650) (Inset x 20,000).

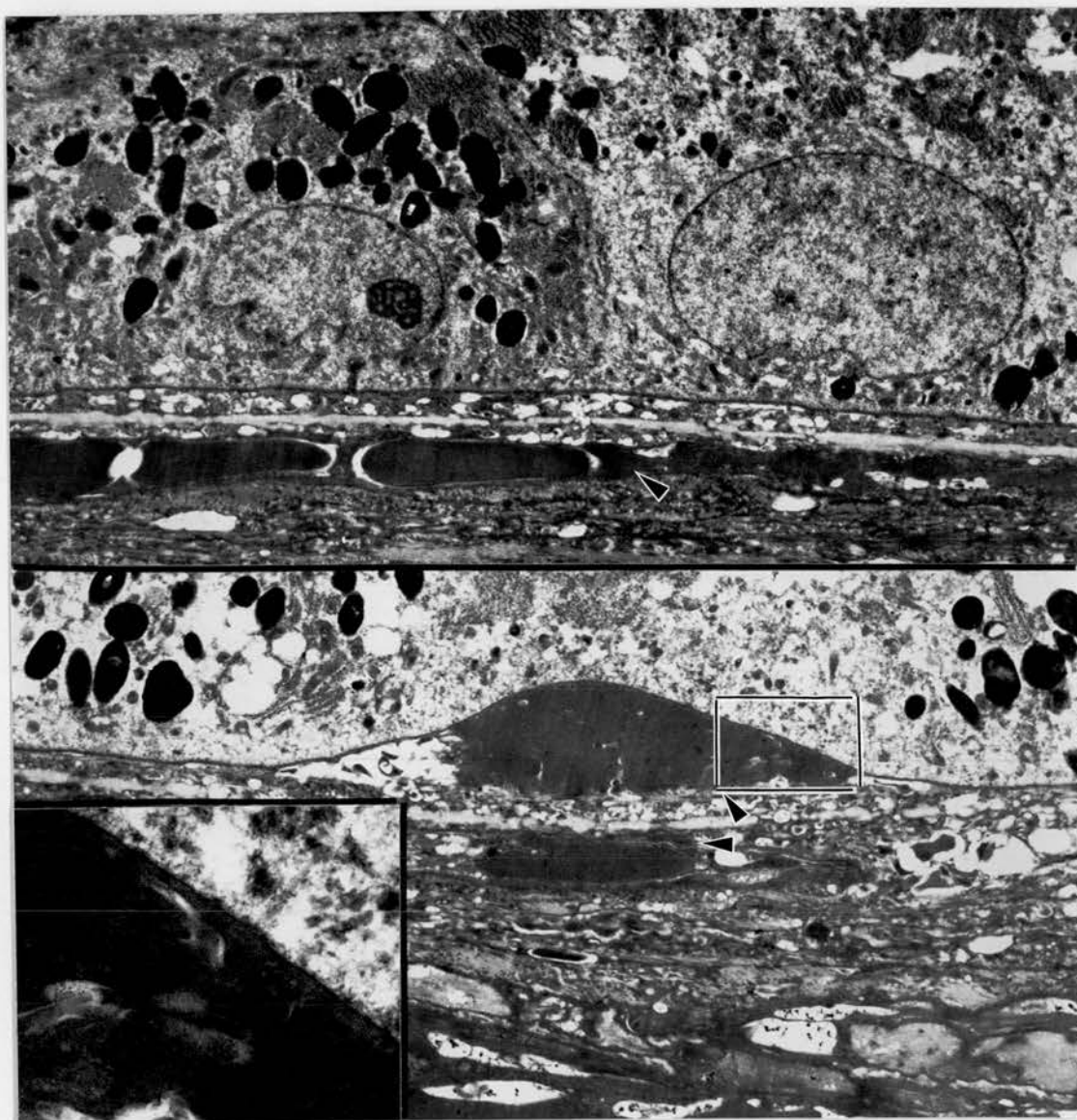


Figure II 51

Discussion

Type II mesangiocapillary glomerulonephritis is a relatively rare, but well recognised condition. The histopathological hallmark is the presence of a linear electron dense deposit in the glomerular capillary basement membrane (136). The pathogenesis is unknown, but there is evidence that an anti-complement antibody is implicated, and the serum complement is characteristically low. However, the situation is complex, and as yet there is no definite knowledge of the nature of the dense material in the basement membrane, though it has been suggested that it represents alteration in a glycoprotein of the basement membrane itself. Immunopathology often shows C_3 almost alone in the capillary walls, as in this case.

In an experimental study of the choriocapillaris, a comparison between the anatomy of the glomerulus and of the choriocapillaris/Bruch's membrane/retinal pigment epithelial complex had been drawn (Chapter III) (256). To my knowledge, this is the first case to be reported of Type II MCGN with clinical and histopathological evidence of ocular involvement, similar to the glomerular lesion.

The results of previous studies of ocular pathology coexisting with glomerular disease are shown in Table II 1.

	Disease State	Conjunctiva	Ciliary Body	Bruch's Membrane	Choroidal Vascular Basement Membrane
JAMPOL, LAHAV, ALBERT, CRAFT 1975 (567)	Goodpasture's disease	-	+	++	++
PERTSCHUK, AZABO, VULETIN, RAINFORD 1977 (909)	Animal model - SLE	-	++	++	++
PERESS, MILLER, PAVU 1977 (902)	Animal model - rat Chronic serum sickness	-	++	-	-
ARONSON, ORDONEZ, DIDDIE, ERNEST 1979 (37)	SLE	+	++	++	++
MUNTEANU 1980 (830)	Monoclonal gammopathy (no histology)	-	-	++	-
NIK, MARTIN, BERLER 1985 (852)	IgA-Kappa Chain monoclonal gammopathy (no histology)	-	-	+	-

Table II 1 Summary of Literature

In a mouse model of SLE (909), the immunoglobulin deposits identified were found in the choroidal vessels, Bruch's membrane, and ciliary processes, as well as in glomeruli, the choroid plexus, and the capillaries at the epidermal-dermal junction. A similar distribution of deposition of immune complexes is found in rat serum sickness (902). However, there are only two reports of deposition of immune complexes in human cases, one describing the postmortem findings in a case of SLE in which the deposits were not detectable clinically (37) and the other a case of Goodpasture's syndrome (antiglomerular basement membrane disease) in which immune complexes were deposited in a uniform linear pattern due to an antibody reaction in the basement membrane). A drusen-like deposit has been described clinically in monoclonal gammopathies (830), and occurs in 21% of patients with SLE (430).

I presume that in this case we report, abnormal material, possibly of immune complex nature, has been derived from the blood, having escaped through damaged vessel walls. Aronson et al (37) found that in their non-hypertensive patients with SLE, there was leakage of fluorescein from atypical drusen into the subretinal fluid under areas of localised retinal detachment.

I would suggest that abnormal deposits in Bruch's membrane and the choroid may not be infrequent in immunologically mediated renal disease, but that they remain unrecognised because the eyes have not been histologically examined. Early this century a case of a patient with chronic

nephritis was reported to have widespread drusen which were interpreted to be exudates from the choroid (1198) but this has been interpreted as hypertensive choroidopathy. Our patient had an episode of accelerated hypertension which was rapidly controlled and did not show the fluorescein angiographic features of RPE damage subsequently.

The patient had a retinal venous occlusion, retinal detachment, and complained of pain during dialysis.

Retinal detachment is a well described complication of accelerated phase hypertension, particularly in toxæmia of pregnancy (377) and in renal failure (657). The cause is thought to be massive exudation of fluid into the choroid with breakdown of the blood/eye barrier (624) probably secondary to RPE infarction (234). This has been demonstrated by fluorescein angiography in which dye is shown to leak out from the choroid into the subretinal fluid (294,420). In all the cases of retinal detachment associated with renal failure in the literature, the patients had marked hypertension (125,268) but the extent of the detachment may be aggravated by haemodilution in patients with renal failure, upsetting the osmotic gradient across the choriocapillaris.

The vascular changes in the choriocapillaris could be attributed to hypertension in this case (41,614), but a deposit such as was described in the stroma of the choriocapillaris and Bruch's membrane has never been previously described. Additional evidence to support my

belief that this is a new phenomenon is that there were no typical infarcts of the pigment epithelium (624).

Retinal venous occlusion may occur in patients on renal dialysis (932,937), probably as a result of raised intraocular pressure. The relative hyperosmolality of the intraocular fluid after dialysis results in an influx of water into the eye under osmotic pressure, with a rise of intraocular pressure of up to 16mm Hg. This phenomenon could also account for the pain of which this patient complained, following episodes of dialysis.

This case demonstrated involvement of the choriocapillaris and Bruch's membrane, and of the renal glomeruli in a similar morphological change; a clinical correlation with drusen-like spots in the fundus is also present. The choriocapillaris/Bruch's membrane/retinal pigment epithelial complex has morphological similarities with the glomerulus. It is likely that both sites are affected by similar disease processes, and that the response of the choroid in renal disease merits further investigation.

4. Conclusion

The study of these three disease states brings to notice several points.

1. The spectrum of age-related changes in the choriocapillaris, Bruch's membrane, and retinal pigment epithelium is broader and more complex than had been previously thought. More detailed clinical pathological correlation of individual cases could continue to contribute to our knowledge (case 1).
2. The diseased retinal pigment epithelium can produce large quantities of extracellular material of different types and distributions (case 2).
3. The choriocapillaris may contribute to the deposition of abnormal materials in Bruch's membrane in the same distribution as is seen in degenerative drusen (case 3).
4. Deposits in Bruch's membrane may excite neovascularization (cases 1,2).
5. The structure of the renal glomerulus has similarities to the structure of the chorioretinal juncture and the two sites may show common disease processes (case 3).